Acta Agronomica Hungarica

VOLUME 40, NUMBERS 1–2, 1991

Akadémiai Kiadó, Budapest

ACTA AGRONOMICA HUNG. HU ISSN 0238–0161

ACTA AGRONOMICA

A QUARTERLY OF THE HUNGARIAN ACADEMY OF SCIENCES

Acta Agronomica publishes papers in English on agronomical subjects, mostly on basic research.

Acta Agronomica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences

H-1117 Budapest, Prielle K. u. 19—35.

Manuscripts and editorial correspondence should be addressed to

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Soil Science and Agrochemistry

PREPARATION OF NATIVE CLAY-HUMUS COMPLEXES FROM SOIL BY CHEMICAL EXTRACTION

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(Received: 5th June 1989; accepted: 13th October 1989)

We used a 0.1 mol aqueous solution of tetramethylammonium hydroxide (TMAH) at room temperature for the extraction of fulvic acid and humic acid-clay mineral complexes from different soils, and peats. By applying TMAH for the extraction could be obtained the humic substances in a nondegradative, native form and a nearly quantitative amount. TMAH neither degrades nor even chemically modifies the humic substances.

The fulvic acids were bound to an Amberlite XAD-8 resin. The humic substances were resolved with aqueous TMAH solution and reprecipitated with hydrochlorid acid, finally resolved with aqueous urea and TMAH solution and reprecipitated for further purification. The average molecular mass of humic substance was examined by gel-chromatography and the structure of humic acid-clay mineral complexes by IR absorption spectroscopy.

By this extraction method the humic acids can be obtained in their native form, i.e. in organominerals, in such a pure state that it becomes possible to examine the structure of these complexes by IR absorption spectroscopy. Studying the IR absorption spectra we can obtain a picture about the structure, i.e. bindings of the organo-mineral clay complexes to humic substances with metal ions which plays a very important role in the formation of soil structure.

Keywords: clay-humus complexes, humic acid, fulvic acid, tetramethyl, ammonium hydroxide, IR-spectra

Introduction

The soil clay-humus complexes play an important role in forming the structure, and in the fertility of agricultural soil, as well as in providing plant nutrients (Stevenson, 1982; Theng, 1974, 1979). The analysis and physico-chemical investigation of these clay-humus complexes are difficult, because during their extraction, these substances are irreversibly degraded to such an extent that it is not possible to prepare them "purely" in their natural state.

The organic material of soils can primarily be divided into living and dead organic substances. The latter constitute the following groups:

— new formations (primary and secondary metabolites of soil microbes).
— non-humic substances (proteins, carbohydrates, waxes, etc.)
— humic substances (the relatively stable and soil-characteristic decay materials of the soil macro- and microorganism and the plant residues of soil).

Previously Achard (1786), Saussure (1804), Sprengel (1826), Berzelius (1839), Mulder (1860) and Hoppe-Seyler (1889) studied the preparation and analysis of the organic humus materials of soil. The research work is rather difficult, due to the great variety and heterogeneity of the organic matter of soil. Even in a relatively uniform and narrow fraction of clay-humus complexes there is little probability that two complexes are identical.

A classical method of separation of humic substances has been applied, based on discontinuous extraction and fractional precipitation of humic acids worked out by Tjurin (1937). These processes are laborious and time-consuming (exhaustive extractions for 18–28 hours and repeated precipitation and filtration), the extraction is not quantitative, finally the humic substances are subjected to a considerable and irreversible degradation.

Several other mild extractants are widespread in the scientific literature for the production of humic substances (Martin-Reeve, 1955; Schnitzer-Wright, 1957; Evans, 1959; Hargitai, 1961; Schnitzer-Skinner, 1968; Khan, 1971; Lakatos et al., 1977). The applied classical digestive agents such as sodium and potassium, as well as ammonium hydroxide and specific organic extractants such as acetyl bromide, primary, secondary and tertiary amines, either lead to irreversible degradation of humic substances or chemical reaction with soil organic matter, therefore, they do not result in a quantitative solubilization of humic acids.

We have elaborated a mild chemical extraction method for solubilization of soil clay-humus complexes in their natural form with tetramethylammonium hydroxide (TMAH) as an extractant. Thus, it becomes possible to examine the structure of these native soil clay mineral-multivalent cation-humic acid complexes (Stevenson, 1982; Theng, 1974).

Materials and methods

We can demonstrate the results obtained by our extraction method in the case of two types of soil and one of peat.

Sample (a) meadow soil of Hosszúhát; total organic material content: 3.0–3.4%, clay content: 44.5%, pH = 5.2–5.4;

(b) calcareous chernozem soil of Nagyhörcsök; total organic material content: 3.0–3.1%, clay content: 23.1%, pH = 7.9–8.0;

(c) lowland peat of Usztatómajor (Keszthely) composed mainly by calcium humate and mixtures of clay minerals, lime-mud, shells and sands; total organic material content 30–70%, ash content: 28–43%, protein content: 12.5%, pH = 7.8.

In our experiments the average samples of soils and peat (50 g) were extracted with a 1 : 1 mixture of methanol-dioxan in order to remove bitumens, waxes, resins, fats, phospholipids etc. and the treated samples were dried in a drying box at 105 °C to a constant weight for 16 hours. After this drying we added a tenfold weight 0.1 mol aqueous hydrochloric acid solution to the dried samples to solve their limecoatings and other acid soluble mineral contaminations. This acidic suspension was kept at room temperature for 24 hours with occasional stirring. In the beginning the pH value of the acidic suspension was pH = 1.0, which gradually rose. We checked the pH value after 1, 2, 5 and 8 hours and acidified the suspension back to value pH = 1. After 8 hours the pH value remained unchanged.

The solid substance was separated by centrifuging and washed with distilled water (50 cm^3).

We added to the washed samples (10 g) 0.1 mol aqueous solution of TMAH (tetramethylammonium hydroxide) as chemical extractant in a quantity of 100 cm^3 for each (1 g) gram of air-dried sample. We kept the suspension at room temperature for 7 days, occasionally stirring it.

After dissolution of the clay-humus complex, the supernatant was separated from the undigested solid residue, then was conc. hydrochloric acid solution added to the supernatant. The suspension was allowed to stand overnight for sedimentation. The well-sedimentated clay-humus complex fractions were separated by centrifuging from the acidic supernatant, which also contains fulvic acids. For the separation of fulvic acids, the supernatant was passed through a 60×2 cm column of Amberlite-XAD 8-type resin at a rate of 360 cm^3/hour, then the resin was eluated by a (9 : 1) solution of acetone —0.1 mol hydrochloric acid. The eluated solution was evaporated in vacuum and the wet fulvic acids were dried above phosphorous pentoxide in a desiccator. Finally they were weighed and analysed.

The clay-humus complex fraction was washed out three times with 200 cm^3 0.1 mol hydrochloric acid solution, and for further purification it was repeatedly solved in 0.1 mol of an aqueous solution of TMAH, up to a value of pH = 7–8. The solution was allowed to stand overnight for sedimentation, then it was centrifuged and filtered, and finally the supernatant was acidified to pH = 1. Thus we obtained a suspension, which was allowed to stand overnight for sedimentation. Then it was centrifuged and washed with 0.1 mol hydrochloric acid. Finally the washed clay-humus complex was freeze-dried and analysed.

For further purification the raw clay-humus complex was treated with urea and a TMAH solution. An aqueous solution of urea was added to the raw substances (40 cm^3 30% solution to 1 g) with constant stirring and the pH value of suspension was adjusted with 0.1 mol TMAH aqueous solution to pH = 7–8. Then the mixture was allowed to stand overnight in order to solve the clay-humus complexes. The insoluble residue was separated by centrifuging with Janetzky K-70 type centrifuge at 15000 g. The filtered supernatant was treated with conc. hydrochloric acid and adjusted to pH = 1. The precipitated clay-humus complex was centrifuged, washed, freeze-dried and kept over phosphorous pentoxide to constant weight.

For comparison the digestion treatment of clay-humus complexes was performed with 0.1 mol sodium hydroxide solution.

The infrared spectra of samples were recorded using a Nicolet 7199 FT-IR absorption spectrophotometer at the resolution of 4 cm^{-1}.

We weighed 0.6 mg clay-humus complex samples, which were compressed into disks after being stirred for 3 min in a vibrator with 250 mg KBr.

Results

The results of the ultimate analysis of fulvic acids are summarized in Table 1. The ultimate analytical data and the yield of clay-humus complexes extracted with 0.1. mol TMAH and NaOH solution can be compared with the aid of data is Table 2. The yield was calculated for C% of soils.

Table 1

Ultimate analytical data of fulvic acids

(1) Fulvic acid sample	C	H	N	(2) ash
	%			
(a)	38.36	5.96	1.01	31.2
(b)	36.85	5.76	1.05	32.9
(c)	59.51	6.07	1.85	—

Table 2

Ultimate analytical data and the yield of clay-humus complexes extracted with 0.1 mol TMAH and NaOH solution

(1)	(2)	C	H	N	OCH_3	(3) ash	(4) yield of clay-humus complex
				%			
(a)	TMAH	19.60	2.80	2.91	0.85	56.80	6.5
	NaOH	18.10	2.61	1.62	0.51	57.94	5.4
(b)	TMAH	18.71	2.80	1.91	0.52	55.51	5.5
	NaOH	18.42	2.22	1.91	0.61	54.49	4.5
(c)	TMAH	55.51	5.61	2.41	4.71	0.84	16.0
	NaOH	54.62	4.62	2.22	4.10	1.37	14.8

* The yield was calculated for C % content of soils

The average molecular mass of clay-humus complex fraction extracted with TMAH was determined by gel-chromatography (Table 3).

The high ash content (Table 2) and the relatively high molecular mass values of soil clay-humus complexes are in accordance with our earlier experiences (Sipos et al., 1974) and indicate that the clay-humus complexes still contain contaminations in a rather large quantity, so these raw substances were submitted for further purification processes.

Table 3

The average molecular mass of raw clay-humus complex extracted with TMAH solution

(1) Sample	Average (2) molecular mass (in kD)
(a)	7.300
(b)	7.300
(c)	9.000

The treatment with an aqueous solution of urea and resolubilization with 0.1 mol TMAH and reprecipitation with hydrochloric acid resulted in a fairly pure clay-humus complex, with ultimate analytical data in Table 4. These clay-humus complexes are suitable for IR absorption study. The IR absorption spectra of these samples can be seen in Figs 1–3.

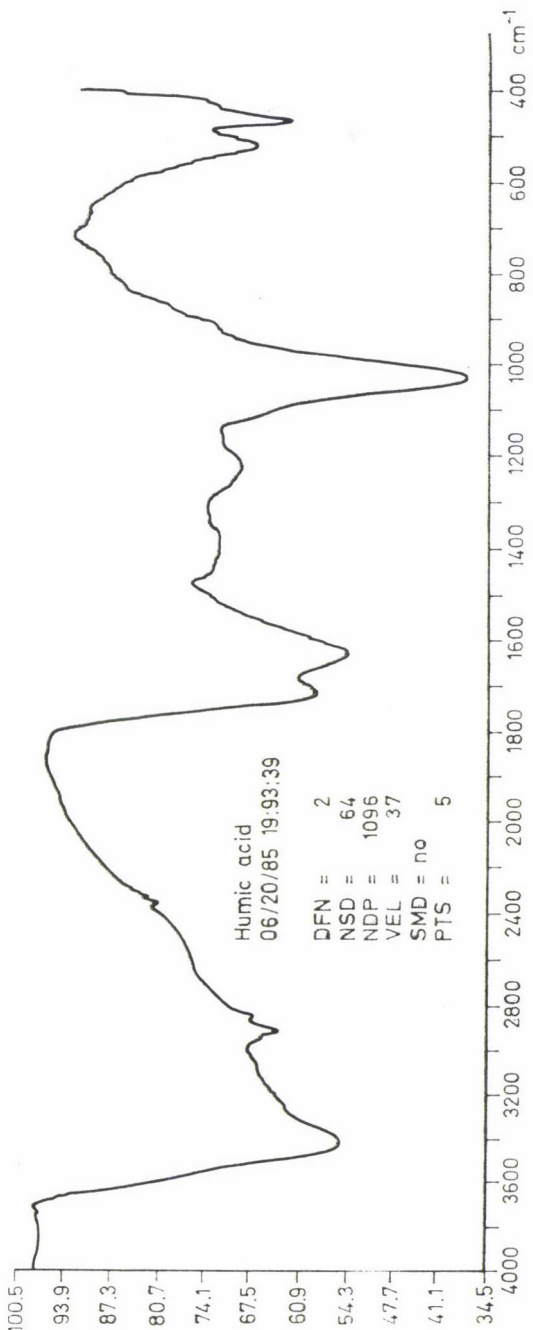

Fig. 1. IR absorption spectra of purified clay-humus complex (sample *a*)

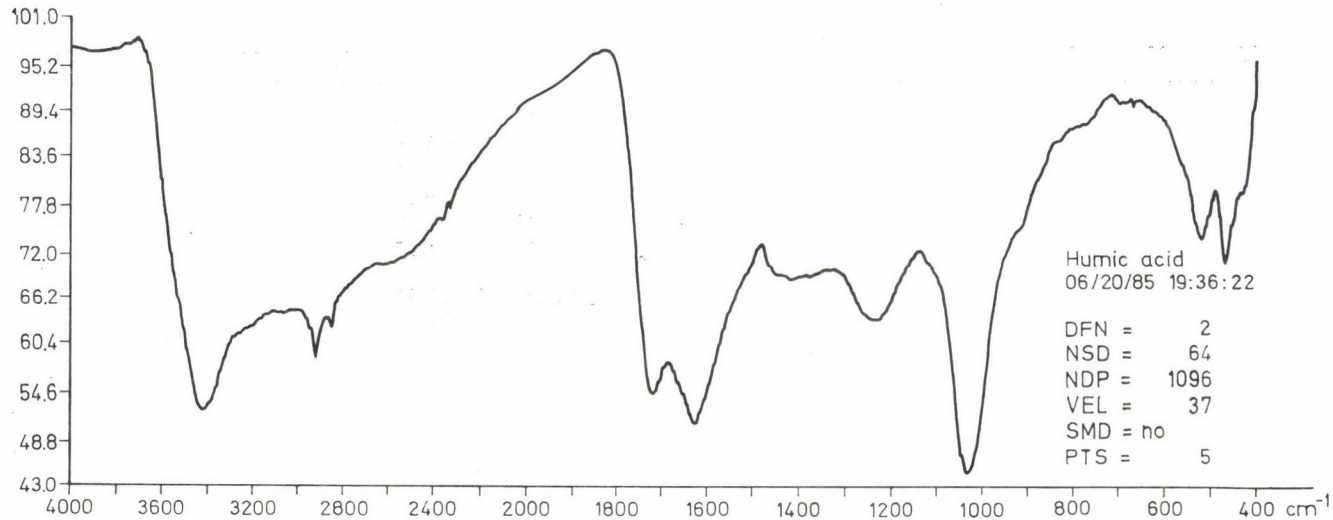

Fig. 2. IR absorption spectra of purified clay-humus complex (sample *b*)

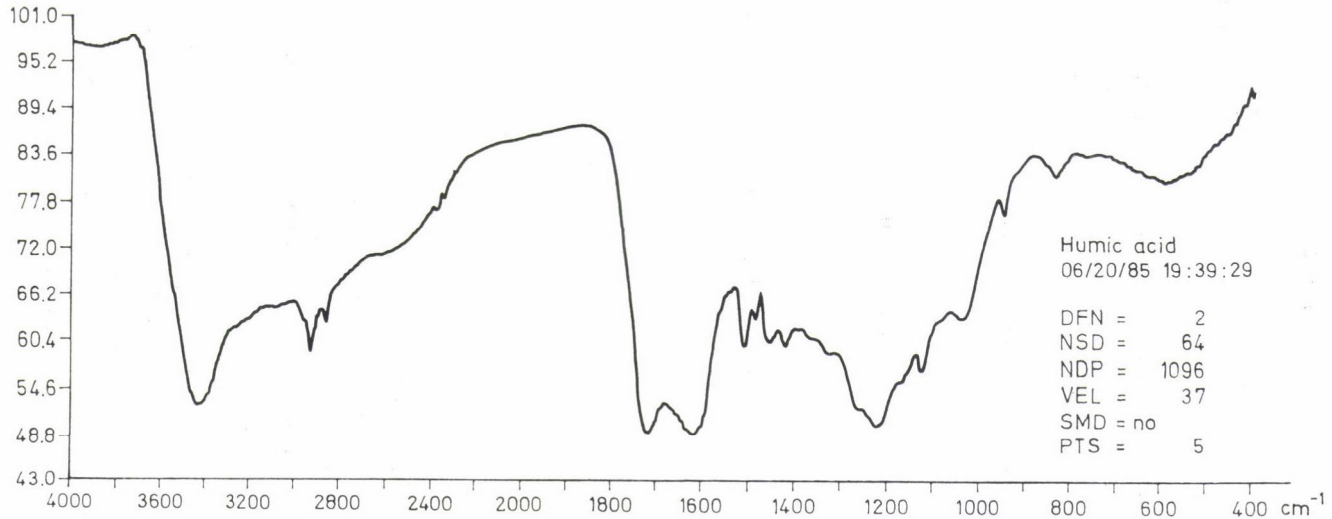

Fig. 3. **IR absorption spectra of purified clay-humus complex (sample *c*)**

Table 4

Ultimate analytical data and yield of purified clay-humus complexes extracted with TMAH

(1) Sample	C	H	N	(2) Ash	(3) Yield of clay-humus complex
			%		
(a)	41.62	3.46	3.06	17.81	21.71
(b)	37.52	3.70	3.31	20.14	21.52
(c)	56.01	4.01	2.21	0.34	92.00

The yield was calculated for C % content of raw humic acids (see in Table 2)

Discussion

Comparing the ultimate analytical data of Table 2 and Table 4, it can be stated that the clay-humus complex fractions obtained with TMAH extraction show relatively low ash- and high carbon content. Evaluating the IR absorption spectra of our samples (Figs 1–3) and trying assignation of characteristic infrared absorption frequencies (Table 5), it renders probable that the clay-humus complex fractions extracted with TMAH and urea correspond to the native clay-humus complexes. The most important IR absorption bands in the spectra (Figs 1–3) which lead to this conclusion are as follows (Table 5):

ν(H. . .OH)	characteristic stretching vibrations of hydroxide (OH) groups associated with hydrogen bridge bonds
$\nu(C_{ar}—H)$	carbon-hydrogen stretching vibrations in aromatic system and
$\nu(CH_3)$ and (CH_2)	carbon-hydrogen asymmetric and symmetric stretching vibrations of methyl and methylene groups
The first ν(C=O)	carbonyl stretching vibrations of saturated aliphatic carboxylic acid dimers, non-conjugated ketones and conjugated esters,
the second ν(C=O)	antisymmetric carbonyl stretching vibration of carboxylate group of metal-carboxylate and carbonyl stretching vibration of conjugated ketones.
$\nu(C_{ar}\cdot\cdot\cdot C_{ar})$	in plane aromatic C=C ring vibrations
$\delta_{as}(CH_3)$ and $\beta_{as}(CH_2)$	antisymmetric deformation C—H vibrations of methyl, methylene and methoxy groups

Finally the very intensive band at 1030 cm^{-1} is mainly originated from Si-O stretching vibration of the clay-humus complexes.

Table 5

Characteristic IR absorption frequencies of purified clay-humus complex samples extracted with TMAH

(1) Band maxima (cm^{-1}) and intensity (T%)						(2)
(a) sample		(b) sample		(c) sample		Assignment
cm^{-1}	T %	cm^{-1}	T %	cm^{-1}	T %	
3421.7	(55.7)	3424.7	(47.2)	3424.9	(38.2)	ν(H . . O—H)
3072.4	(68.7)	3072.1	(60.0)	3066.0	(59.0)	$\nu(C_{ar}—H)$
3007.4	(70.2)	3004.7	(60.9)	3006.2	(62.3)	
2959.0	(65.9)	2959.0	(62.4)	2959.0	(58.2)	
2923.4	(66.3)	2924.2	(55.3)	2924.7	(62.5)	$\nu(CH_3)$ and $C(H_2)$
2854.0	(70.0)	2853.9	(59.1)	2852.0	(62.5)	
1718.6	(58.8)	1718.4	(50.7)	1718.4	(48.8)	ν(C=O)
1626.6	(53.8)	1623.8	(47.2)	1618.8	(46.9)	ν(C=O)
~1508.0	(71.6)	~1516	(63.3)	1512.5	(61.1)	$\nu(C\cdots C)_{ar}$
		~1466	(64.0)	1487.4	(65.3)	$\delta_{as}(CH_3)$
~1458.0	(70.7)	~1444	(62.6)	1454.3	(61.1)	$\beta_{as}(CH_2)$
1417.9	(69.3)	1415.8	(62.0)	1421.3	(61.1)	$\nu(C\cdots C)_{ar} + \beta(C_{ar}—H)$
1388.6	(69.0)	1392.7	(61.9)	1389.8	(63.6)	$\delta_s(CH_3)$; $\beta_s(CH_2)$
1378.0	(68.9)	1377.7	(64.9)	~1378.0	(62.8)	
1368.0	(69.2)	1368.0	(62.06)	~1368.0	(62.4)	
				~1356.0	(62.0)	
1335.4	(69.9)	1334.8	(64.2)	1327.9	(60.0)	ν(C—C)
~1260	(65.7)	—		1262	(52.6)	ν(C—C)
1224.3	(64.3)	1231.7	(55.5)	1221.6	(49.9)	γ(CO)
1163.4	(66.2)	—		1173.2	(56.0)	β(=CH)
1152.6	(66.2)	~1125	(61.0)	1126.2	(57.5)	β(=CH) + γ(CO)
~1090	(55.3)	~1090	(57.8)	~1090	(64.8)	γ(CO) + β(COC)
1031.5	(33.3)	1028.9	(38.0)	1036.2	(64.5)	ν(Si—O) + β(=CH) + γ(CO)
~914	(63.9)	~914	(62)	948.9	(79.8)	
~872	(69.2)	~872	(66.4)	—		
846	(73.3)	831.3	(69.4)	835.1	(84.7)	$\gamma(C_{ar}—H)$
797.3	(75.1)	797.8	(79.3)	—		
770.8	(75.5)	768.8	(70.2)	768.2	(87.5)	
665.9	(74.8)	665.8	(70.6)			
521.7	(53.4)	521.1	(55.8)	593.7	(80.8)	γ(O—H)

These results are supported by our earlier IR absorption studies of humic acids (Lakatos et al., 1977; Vinkler et al., 1975).

Summary

We used a 0.1 mol aqueous solution of tetramethylammonium hydroxide (TMAH) at room temperature for the extraction of fulvic acid and clay-humus complexes from different soils, and peats. By applying TMAH for the extraction we could obtain the humic substances

in a nondegradative, native form and a nearly quantitative amount. In contrast with ammonium hydroxide and the primary, secondary and tertiary alkylamines, TMAH neither degrades nor even chemically modifies the humic substances.

The fulvic acids were bound to an Amberlite XAD-8 resin. The clay-humus complexes were resolved with aqueous TMAH solution and reprecipitated with hydrochlorid acid, then finally resolved with aqueous urea and TMAH solution and reprecipitated for further purification. The average molecular mass of clay-humus complexes were examined by gel-chromatography and the structure of clay-humus complexes by IR absorption spectroscopy. From Table 2 it can be seen that the raw clay-humus complexes obtained by 0.1 mol TMAH extraction have lower ash and higher carbon and nitrogen content than the samples which were extracted with 0.1 mol sodium hydroxide. The relatively high ash content and the molecular mass in Table 3 refer to the presence of different contaminations. The analytical data of the purified clay-humus complexes can be seen in Table 4. These purified clay-humus complexes are already suitable for recording IR absorption spectra for structure determination (Figs 1–3). The assignment of characteristic IR absorption frequencies of samples can be found in Figs 1–3.

By this extraction method the soil clay-humus complexes can be obtained in their native form, i.e. in organominerals, in such a natural state that it becomes possible to examine the structure of these complexes by IR absorption spectroscopy. Studying the IR absorption spectra we can observe the structure of clay-humus complexes which play a very important role in the formation of soil.

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Acta Agronomica Hungarica, Vol. 40 (1—2), pp. 13—30 (1991)

EXTRACTION AND FRACTIONING OF SOIL HUMIC ACIDS BY ELECTROELUTION

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(Received: 8th June 1989; accepted: 13th October 1989)

For the non-degradative extraction of organic matter from soils, peats and humic manures an electroelution apparatus combined with membrane separation electrodialysis has been constructed, by means of which in properly chosen aqueous buffer solutions, at room temperature, with the aid of a suitable membrane filter, fractions suitable for analysis and structure examination can be obtained with nearly quantitative output. The routine variation of this apparatus is suitable for quick routine analyses of a larger number of the above samples. The paper describes the use of the apparatus, and characterizes the initial samples with the help of the organic matter fractions obtained by the method.

Keywords: brown forest soil, "Biovegetal", "Cofuna", electroelution, electrodialysis, fulvic acid, sandy soil, humic acid, meadow chernozem, meadow soil, acid sandsoil, fen-peat

Introduction

In our previous paper (Buzás et al., 1989) a mild extraction of humic acid-clay mineral complexes and humic substances from arable soils and peats with an aqueous solution of tetra-methyl-ammonium-hydroxide was described. In this work the extraction of organomineral complexes and native humic acids by a similar non-degradative electroelution method combined with membrane separation electrodialysis is discussed.

The electroelution is a combination of two separation methods: the electrophoresis and the simple washing-off, i.e. elution (Morris-Morris 1963). In electrophoresis (ionophoresis) electrically charged macromolecules, colloidal or coarse suspension particles in a liquid of high dielectric constant, under the influence of a homogenous electric field created by direct current move towards the electrodes (anaphoresis or cataphoresis). Separation by electrophoresis is generally carried out in neutral, mildly acidic or basic aqueous buffer solution at room temperature, with low ion intensity, in the course of cooling. Besides this so-called free electrophoresis carried out in liquids electrodialysis and carrier electrophoresis are also used to avoid gravitation convections. In electrodialysis the anode and cathode chamber is separated by a semipermeable membrane. In carrier electrophoresis the carrier can be: paper, asbestos, cotton-cellulose, cellulose-acetate, glass-dust, glass bead, agargel, starch, synthetic resin, polyacrylamide, silk, etc.

Substances separated by electrophoresis are simply washed off the carrier — eluated — into the aqueous buffer solution.

Since 1950 (Gordon et al. 1950) electroelution has been widely used to separate biopolymers, such as proteins, protein-metal complexes, acidic polysaccharides, nucleic acids and their degradation products for analytical and preparative purposes (Smith 1980, Lee-Sinsheimer 1974, Allington 1978). After the electrophoresis the individual fractions get into different parts of the separating gel. From the adequate pieces of the gel the macromolecules are often obtained by electrodialysis (Raymond 1964, Zassenhaus et al. 1982, Jacobs-Clad 1986, An der Lan et al. 1983, Hashizume et al. 1984).

There is a preparative gel-electrophoresis method in which the macromolecules passing through the separating gel enter into a liquid current which continuously removes them from the apparatus, whereby the separation and analysis of each molecule fraction becomes possible (Mann–Huang 1969). The separation of the different molecule fractions can also be solved by electrodialysis following electroelution (Morris-Morris 1963) using semipermeable membranes of different pore size.

It became known even at the beginning of the century that humic acids extracted from soils and the humate ions in electric field move to the anode (Odén 1912). Later it was found that, with the exception of alkali-metal- and alkali-earth metal humates, the metal humates also move to the anode. The reason is that, while alkali-metal- and alkali-earth metal ions are linked with the humate anions by ionic bond, other metal ions are linked with them by a strong complex bond (Kleist 1963). On the basis of the electrophoretic phenomenon observed various humus separation methods have been elaborated: paper electrophoresis (Flaig et al. 1975), polyacrylamide gel-electrophoresis (Gonsalez–Hubert 1972, Klöcking 1973, Castagnola et al. 1978, 1979, Mora de Gonsalez 1981), electrofocussing method (Cacco et al. 1974, Gjessing–Gjerdahl 1972), isotactophoresis (Curvetto et al. 1974, Curvetto–Orioli 1982, Orioli–Curvetto 1980), electrodialysis (Desai 1980, Dormaar 1967, Löddesöl 1932). By the help of these methods, fractions of different electrophoretic mobility could be separated: fulvic acid, himatomelic acid, brown and grey humic acid. However, the highly heterogeneous nature of humic substances made the use of electroseparation methods for the separation of humic substances very difficult. Thus, Thornton (1975) queried utility of isoelectric focussing and isotacophoresis. Owing to the high sensitivity and heterogenity of humic substances the newer methods like affinity electrophoresis (Andrews 1988) or electroultrafiltration (Németh 1976) are also unsuitable for the extraction and separation of the humus compounds of soil in native form.

To extract the soil humic acids in native form and separate them a new method was elaborated by combining electroelution and electrodialysis.

Materials and methods

The following samples were analysed:

(a) Acidic sandsoil from Nyírlugos
(b) Brown forest soil from Keszthely
(c) Brown forest soil from Putnok
(d) Chernozem from Kompolt
(e) Chernozem from Mezőhegyes
(f) Chernozem from Nagyhörcsök
(g) Meadow chernozem from Mosonmagyaróvár
(h) Meadow soil from Hosszúhát
(i) Meadow soil from Iregszemcse
(j) Calciferous sand soil from Kecskemét
(k) Fen peat from Keszthely
(l) BIO-VEGETAL "humus manure" from Tersanpuglia, Italy (85% olive seed-cake, marc of grapes, straw, sugar-beet slices, leaves, 15% inoculum)
(m) COFUNA "humus manure" from the Badacsony State Farm, Aszófő (70% marc of grapes, 20% poultry manure, 10% inoculum)
(n) Calcareous sandsoil from Őrbottyán

The organic matter content of the samples was determined by the chromate method (MSZ 21470/5283, MSZ-08-0210-77, MSZ-08-0452-80). By this method the organic matter contained in the soil sample is oxidized with a known quantity of surplus $K_2Cr_2O_7$, then the $K_2Cr_2O_7$ in excess is titrimetrically — and the chromate ions produced are colorimetrically determined. The method was calibrated with glucose. In this way, the combined amount of all oxidizable material present in the soil is obtained.

The organic matter content was determined as follows:

0.05–0.2 g ground air-dry sample passed through a sieve of 0.5 mm mesh was measured into a test-tube and suspended with 0.3 cm^3 of distilled water. From the soil extracts, 1 cm^3 was measured into the test-tube, and a distilled water solution of 1 cm^3 1/6 mol/dm^3 potassium dichromate and 2.0 cm^3 concentrated sulphuric acid were added. The solution was carefully shaken, and heated at 120 °C in a test-tube thermostat for 20 minutes. When cooled, it was diluted with 10 cm^3 of distilled water (when analysing soil extracts dilution was not performed) and the concentration of the chromium (III)-ions was photometrically determined with a Spektromom 202 apparatus at $\lambda = 590$ nm wavelength. For the preparation of the calibration curve 2.75 g crystalline glucose ($C_6H_{12}O_6 \cdot H_2O$) was dissolved in 1000 cm^3 of distilled water and from this various quantities were measured 1 cm^3 of the solution prepared so corresponds to 0.01 g C or to 0.0171 g of organic matter; that is, humus.

As a measure of the humification of humic substances dissolved in the soil extracts the colour quotient, the $Q = \frac{E_{465}}{E_{665}}$ extinction quotient was used (Welte 1955). This value decreases with the increase of the rate of humification, i.e. condensation, and with the increase of the molecular weight (Schnitzer-Khan 1972, Stevenson 1982, Aiken et al. 1985). The value of Q is 6.0–8.5 for fulvic acids, 5.0–5.5 for brown humic acids and 2.2–2.8 for grey humic acids; 5.0 for podzolic soils, 3.0–3.5 for chernozems, and roughly 3.5 for grey forest soils. Measurements were carried out in a 0.1 mol/dm^3 sodium-pyrophosphate and 0.07 mol/dm^3 phosphoric acid buffer solution of pH 7.0. The values obtained were higher than what we should have obtained in a 10 cm^3 0.05 mol/dm^3 aqueous solution of sodium hydrocarbonate according to Chen, Senezi and Schnitzer (1977) (see Table 1).

For the sake of comparison with the electroelution (EE) method which we elaborated, three different extractives were used for the extraction of the humic substances of the samples: 0.1 mol/dm^3 sodium-hydroxide (pH = 13; method I), 0.1 mol/dm^3 sodium-pyrophosphate (pH = 10; method II) and 0.1 mol/dm^3 sodium-pyrophosphate and 0.07 mol/dm^3 phosphoric acid buffer (pH = 7.0; method III). The electroelution extraction was also carried out in the above sodium pyrophosphate-phosphoric acid buffer of pH 7 (Table 1).

Considering that the quantity of the sample to be measured was very small, the extent of error originating from the inhomogeneity of the sample was examined. From samples of 1000 g collected considering the rules of field sampling and prepared as prescribed, 3×50 g sample parts were taken out and ground further using an electric grinder. From the 50 g parts 0.2 g was weighed in each case. The average values of the measuring results and the maximum and minimum deviations from them are shown in Table 1. It can be established from Table 1 that a representative soil sample can be well characterized by the method described.

Description of the electroelution apparatus developed for research purposes

The electroelution-electrodialysis apparatus suitable for the non-degradative extraction of organic material from soils, peats and artificial plant culture media, and for a scientific level determination of organic material composition (Mády et al. 1984) consists of three main parts (Fig. 1.): the anode chamber, the

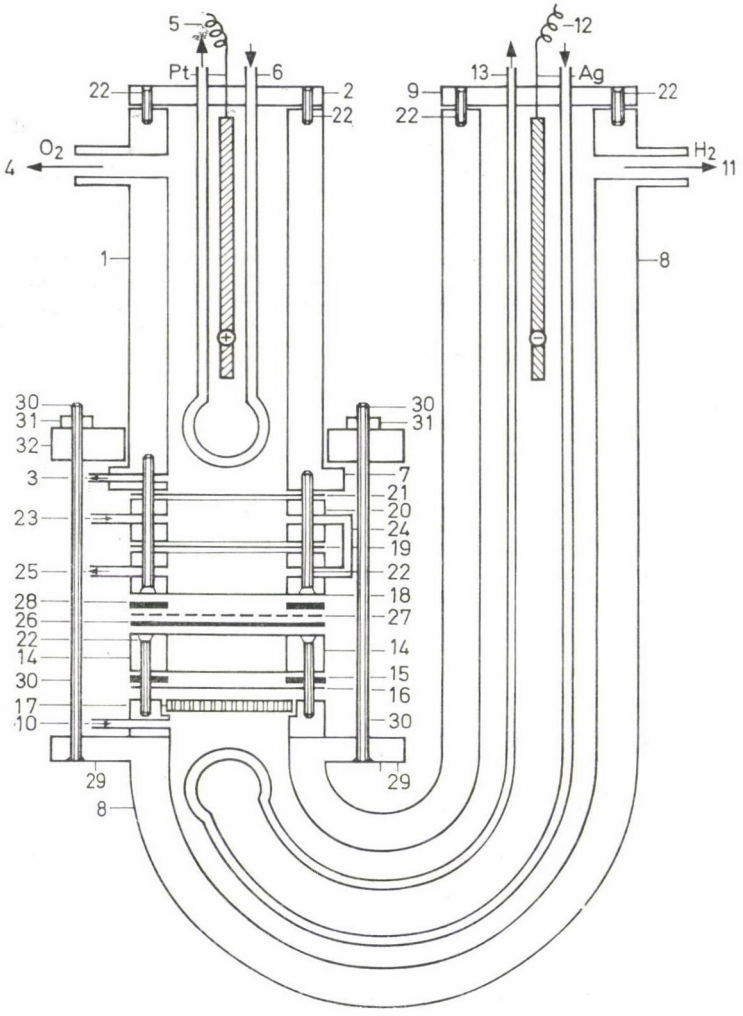

Fig. 1. Electroelution apparatus for research purposes (1) anode chamber, (2) cover plate, (3) lower entrance branch, (4) upper outlet branch, (5) Pt-electrode, (6) glass cooling pipe, (7) rim, (8) cathode chamber, (9) cover plate, (10) lower entrance branch, (11) upper outlet branch, (12) Ag-electrode, (13) thin-walled plastic cooling pipe, (14) sample chamber, (15) packing plate, (16) cellophane layer, (17) porous filter layer, (18) lower elution chamber, (19) cellophane layer, (20) upper elution chamber, (21) cellophane layer, (22) plastic screws, (23) entrance branch, (24) transfer pipe, (25) outlet branch, (26) special filter layer, (27) synthetic screening cloth, (28) packing plate, (29) rim, (30) through-bolt, (31) nut, (32) plastic clamping plate

cathode chamber and the sample- and elution chambers between them. In the anode chamber (1) there is the Pt-electrode (5), the cooling of which is provided by a glass cooling pipe (6). The Pt-electrode and the glass cooling pipe are fixed by the cover plate (2) by means of plastic screws (22). Through the lower entrance branch of the anode chamber (3) a buffer solution is introduced continuously, which leaves through the upper outlet branch (4). To the bottom of the anode chamber the upper (20) and lower elution chambers (18) are attached by plastic screws (22). Between the anode chamber and the upper elution chamber, as well as between the upper and the lower elution chamber, a cellophane layer is placed (21 and 19, resp.). In the elution chambers a buffer solution flow is generated through the entrance branch (23), the transfer port (24) and the outlet branch (25). The Ag-electrode is placed in the cathode chamber (12); it is cooled by a thin-walled plastic cooling pipe (13). The Ag-electrode and the plastic cooling pipe are fixed to the cover plate (9) by plastic screws (22). At the bottom of the "J"-shaped cathode chamber there is a porous filter bed (17) which supports a celophane membrane (16). Above the cellophane that serves to prevent the flowing of liquid, the sample chamber (14) is fixed by plastic screws (22) with a packing plate inserted. In the cathode chamber through the lower entrance branch (10) buffer solution is introduced which leaves through the upper outlet branch (11). The soil sample is placed in the sample chamber, then the chamber is filled with buffer solution. It is covered by the special membrane filter layer (26), and above it the synthetic screening cloth (27), then the packing plate (28) are placed. The mounted anode chamber is placed onto the mounted cathode chamber, then the rims (7, 29) are pressed to each another by means of a through bolt (30), a nut (31) and a plastic clamp plate (32), until completely sealed.

The special membrane filter layer ensures the separation of the humic acids from the larger organic and inorganic colloids. The humic acid fraction to be examined is washed out of the elution chambers with a buffer solution current perpendicular to the direction of the electrophoresis, and gathered for the purpose of analysis.

How to use the electroelution apparatus developed for research purposes

When working with the apparatus the measuring system shown in Fig. 2. was used which contains the following units: electroelution apparatus (I), reagent solution container (II), multichannel chromatography pump (III), fraction collector (IV), electric supply unit (V), ultra-thermostat (VI).

The electroelution apparatus (Fig. 1.) is assembled as follows: the Pt-electrode (5) and the glass cooling pipe (6) are placed in the anode chamber (1), then fixed with the cover plate (2) and the plastic screws (22). To the bottom of the anode chamber a cellophane layer (21) is fitted, the upper elution

Table I

Comparison of the quantities of organic matters

Sample	Total organic matter content mg C g^{-1}	Quantity of organic matter extractable by various solvents, mgC · g^{-1} soil; I 0.1 M NaOH (pH = 13) 24 hours mg C g^{-1}	II 0.1 M $Na_4P_2O_7$ (pH = 10) 24 hours mg C g^{-1}	III 0.1 M $Na_4P_2O_7$ 0.07 M H_3PO_4 (pH = 7.0) 3,5 hours mg C g^{-1}
1.	2.	3.	4.	5.
a) Acidic sandsoil	3.62	0.285 +0.132	0.571 +0.023	0.685 +0.037
Nyírlugos		—0.132	—0.034	—0.051
b) Brown forest s.	10.51	0.815 +0.093	1.869 +0.096	1.009 +0.010
Keszthely		—0.130	—0.127	—0.010
c) Brown forest g	13.65	1.877 +0.146	3.259 +0.265	1.979 +0.085
Putnok		—0.189	—0.189	—0.059
d) Chernozem	19.41	2.815 +0.079	5.129 +0.217	2.807 +0.136
Kompolt		—0.097	—0.239	—0.109
e) Chernozem	27.46	1.126 +0.291	5.032 +0.136	3.080 +0.068
Mezőhegyes		—0.294	—0.102	—0.103
f) Chernozem	21.80	1.021 +0.296	3.505 +0.034	2.270 +0.047
Nagyhörcsök		—0.290	—0.034	—0.031
g) Meadow chernozem	10.91	0.734 +0.146	1.328 +0.109	0.895 +0.063
Mosonmagyaróvár		—0.142	—0.083	—0.084
h) Meadow soil	23.40	1.915 +0.202	5.020 +0.097	2.480 +0.068
Hosszúhát		—0.192	—0.162	—0.071
i) Meadow soil	17.73	0.865 +0.077	2.356 +0.027	1.524 +0.050
Iregszemcse		—0.038	—0.021	—0.046
j) Calc. sandsoil	2.49	0.521 +0.041	0.302 +0.083	0.396 +0.077
Kecskemét		—0.049	—0.075	—0.066
k) Fen peat	304.9	142.05 +0.096	110.72 +1.42	69.01 +0.27
Keszthely		—0.190	—1.42	—0.27
l) BIO-VEGETAL	240.2	23.50 +1.17	26.17 +0.69	16.44 +0.12
		—1.19	—0.62	—0.23
m) COFUNA	336.5	69.46 +1.21	40.42	22.58 +0.18
		—1.45		—0.16

extracted by various techniques

Colour quotient (Q), quantity (mg C · g^{-1})
(0.1 M $Na_4P_2O_7$ + 0.07 M H_3PO_4 pH = 7.0 buffer; 80 V; 3,5 hours

Whatman GF/B fulter humic acid + fulvic acid				Kalle cellophane filt. fulvic acid			
$Q = \frac{E_{465}}{E_{665}}$	mg C · g^{-1}		% to the amount extracted by method III	$Q = \frac{E_{465}}{E_{665}}$	mg C · g^{-1}		Humic acid/ fulvic acid ratio
6.	7.		8.	9.	10.		11.
5.89	0.647	+0.194 —0.133	94.58	10.40	0.518	+0.227 —0.138	0.24
5.61	1.839	+0.151 —0.213	182.29	11.81	0.9618	+0.171 —0.127	0.893
6.11	2.766	+0.534 —0.135	139.82	17.38	1.486	+0.109 —0.162	0.8613
5.49	3.984	+0.658 —0.538	142.00	10.73	1.407	+0.095 —0.114	1.8313
5.52	3.880	+0.272 —0.169	125.73	14.48	1.715	+0.337 —0.227	1.262
5.65	3.508	+0.376 —0.219	154.26	13.84	1.586	+0.321 —0.090	1.211
4.70	1.354	+0.193 —0.237	151.28	10.66	0.855	+0.144 —0.147	0.583
5.05	3.310	+0.120 —0.080	133.52	12.55	1.222	+0.178 —0.176	1.708
5.90	2.346	+0.142 —0.103	154.01	14.16	1.232	+0.293 —0.183	0.904
5.61	0.301	+0.008 —0.007	76.10	—	0.306	+0.143 —0.101	—
7.50	59.94	+5.39 —4.46	86.85	9.68	28.62	+2.74 —0.84	1.094
7.66	15.12	+0.99 —0.72	90.86	10.28	7.86	+0.525 —0.679	0.923
9.02	23.54	+2.07 —0.76	104.25	12.07	9.78	+0.551 —0.303	1.406

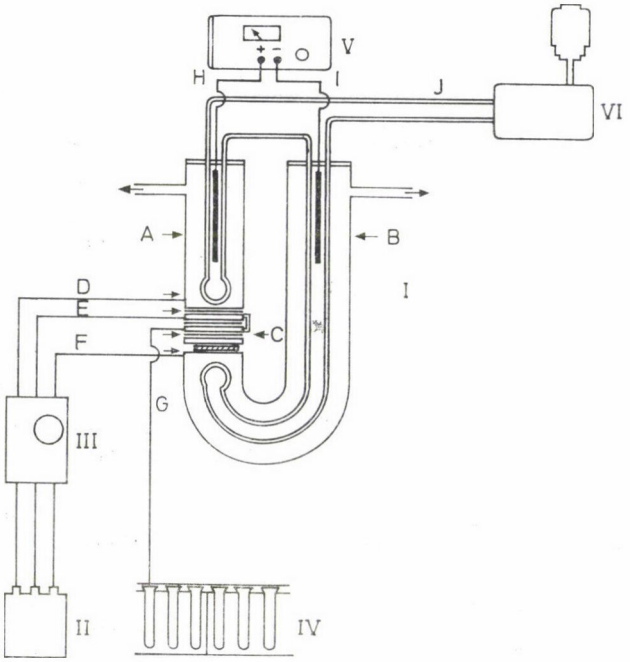

Fig. 2. Measuring system for the electroelution apparatus (I) electroelution apparatus, (II) reagent solution container, (III) multichannel chromatography pump, (IV) fraction collector, (V) electric supply unit, (VI) ultrathermostat, (A) anode chamber, (B) cathode chamber, (C) special filter layer, (D, E, F) connecting silicone rubber pipes, (H, I) connecting electric lines, (J) cooling pipe joining the ultrathermostat

chamber (20), and a second cellophane layer (19) are placed on it. Finally the lower elution chamber (18) is put on this. The elution chambers (18, 20) are fixed to the anode chamber fluid-tight by plastic screws (22). Proper sealing is ensured by the cellophane layers (19, 21). The Ag-electrode (12) and the thin-walled plastic cooling pipe (13) are placed in the cathode chamber (8), then fixed by the cover plate (9) and plastic screws (22). The cathode chamber is filled with buffer solution up to the lower rim, the porous filter layer (17) is placed on the lower rim of the cathode chamber, then the cellophane layer (16) and the packing plate (15) are put on it. Care must be taken that bubbles do not remain in the apparatus as they disturb the measurements. The sample chamber (14) is placed on the packing plate and fixed fluid-tight by plastic screws (22). The entrance branches (3, 10, 23) are connected with the multichannel chromatographic pump by silicone rubber pipes (D, E, F). By the help of this the anode chamber, the cathode chamber as well as the lower- and upper elution chambers are filled bubble-free with the buffer solution. The outlet branch (25) is connected with the fraction collector by a silicone rubber pipe (G). The chromatographic pump and the fraction collector are adjusted so that 4 cm^3 buffer solution is collected in each test tube every 15 minutes.

One g sample is measured into a small measuring cup then wetted with 100 μl reagent solution and left standing for 30 minutes. After 30 minutes the sample is washed quantitatively into the sample chamber, then the special membrane filter layer (26), the synthetic screening cloth (27) and the packing plate (28) are placed on the chamber bubble-free. By means of the rims (7, 29), the throughbolt (30), the nut (31) and the plastic clamping plate (32) the mounted anode chamber is fixed to the mounted cathode chamber.

The coolers (6, 13) are connected with a MLV-2 ultrathermostat (VI) by silicone rubber pipes (J). In the course of measuring, the apparatus is kept at 24 °C by the thermostat. The electrodes are connected by lines (H, I) with a Labor-MIM type stabilized electrical source (V). When repeating the measurement only the anode- (1) and cathode chambers (8) must be dismounted, the sample chamber cleaned and the special membrane filter (26) replaced.

Description of the electroelution apparatus developed for routine analyses

For the quick determination of free and weakly bound humic substance fractions, a routine apparatus was developed which is suitable for examining several soil samples at a time (Mády et al. 1987).

In the routine electroelution apparatus (Fig. 3.) separating tubes (1) (maximum 10) are placed vertically between the cathode- (K) and the anode (A) chamber. Both chambers contain the same buffer solution (pH = 7–10). In order to prevent the flowing of liquid the bottoms of the separating tubes which sink into the anode chamber are sealed by a porous filter plate (2) with a cellophane layer (3) placed on it, which is fixed by a rubber ring (4). The separating tubes aref illed with an inert carrier (5), e.g., quartz, glass, plastic or starch powder, — which does not adsorb the humic substances — suspended in the buffer solution filling out the electrode chambers. The upper ends of the separating tubes (1) are fixed in plastic heads (6) by screws (7) and guard-rings (8). The upper end of each separating tube in the plastic head is covered with a porous plate (9). On the upper surface of this, the controlled pore-size membrane filter layer (10) fixed by a plastic ring (11) is placed. The 0.1–0.2 g soil sample (S) suspended in the buffer solution is filled in the cavity of the plastic ring. The suspended soil sample is covered by the porous layer (12). The separating tubes containing the sample are put in the holes (13) formed at the bottom of the cathode chamber, where they are fixed and made fluid-tight by rubber rings (14).

When the current (40–100 V) is switched on, the anionic components of the soil sample move towards the anode. Passing through the filter (10) they go into the buffer solution among the inert carriers filling the separating tube. Separation of the humic substances takes 3–4 hours. Work can be done with 10 tubes simultaneously.

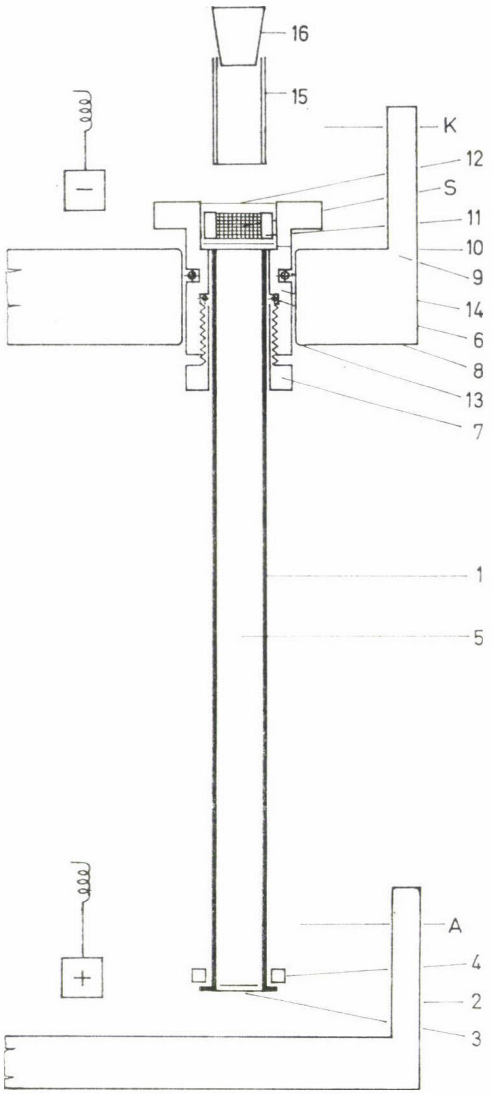

Fig. 3. Routine electroelution apparatus. (A) anode chamber, (K) cathode chamber, (S) soil sample, (1) separating tube, (2) porous filter, (3) cellophane layer, (4) rubber ring, (5) inert carrier, (6) plastic head, (7) screw, (8) insulating ring, (9) porous filter bed, (10) controlled membrane, (11) plastic ring, (12) porous layer, (13) hole for the separating tube, (14) rubber insulating ring, (15) glass feeding pipe, (16) rubber plug

After switching off the current the separating tubes are removed from the apparatus, then taking out the soil sample, the filter layer (10) and the cellophane layer (3) which prevents the flow of liquid, the separated substances can be washed out of the inert carriers present in the tube by flowing buffer solution.

How to use the routine electroelution apparatus

The clamping screw (7), then the packing ring (8), are pulled over the empty separating tube, and the latter is placed in the hole of the plastic head (6) so, that its upper rim should be at the same level where the lower opening of the plastic head is, then the clamping screw (7) is tightened. In the plastic head a thick-walled silicone rubber ring (11) is fitted in which a small glass tube (15) is placed. Through the glass tube inert filling material suspended in buffer solution is fed into the separating tube by a pipette. The surplus buffer solution leaves through a porous filter layer (2) at the bottom of the separating tube. When the separating tube has filled up with an inert carrier and the surplus has accumulated in the feeding tube (15), the tube is closed with a rubber plug (16) and left standing for about 2 hours in order to compact the inert carrier. Then at the bottom of the separating tube a cellophane layer (3) preventing the outflow of the liquid is placed bubble-free and fixed with a rubber ring (4). From the plastic head the feeding glass tube (15) and the silicone rubber ring (11) are lifted out, and the superfluous inert carrier is removed by the help of a spatula. In the cavity of the plastic head some buffer solution is pipetted, the porous filter plate (9) and the controlled pore-size membrane layer (10) are placed, bubble-free then fixed with the aid of a silicone rubber ring (11).

From the cavity of the silicone rubber ring the superfluous buffer solution is removed and the previously measured 0.1–0.2 g soil sample (S) is placed in it through a small funnel. Then the soil sample is wetted with the buffer solution using a syringe. The separating tubes containing the soil samples prepared so are placed in the holes (13) formed in the bottom plate of the cathode chamber, then the cavity of the silicone rubber ring containing the sample in the separating tubes is carefully filled with buffer solution by the help of a syringe. Then it is covered bubble-free with a porous filter layer (12). Following this the anode- and cathode chambers are filled up with buffer solution and the direct current is switched on. Using 10 separating tubes, in case of 80 V a current intensity of 150–180 mA passes through the apparatus (15–18 mA/tube, that is 1.2 W hour). The electroelution is carried on for some 3.5 hours, and during this time the humic acids flowing out from the soil samples stain about three-quarters (9 cm in length) of the separating tubes yellowish brown. Then the direct current is switched off, the buffer solution is removed from the cathode chamber and the separating tubes are lifted out of the apparatus. The covering porous layer (12) is removed, then the soil sample is washed out from the cavity of the silicone rubber ring (11), with buffer solution and the membrane (10) is removed with a needle. The glass feeding tube (15), which is filled with buffer solution and closed with a rubber plug (16) is placed in the cavity of the silicone ring. The cellophane layer (3) preventing the outflow of the liquid at the bottom of the separating tube is removed, and by pulling out the rubber plug the flow of liquid is started.

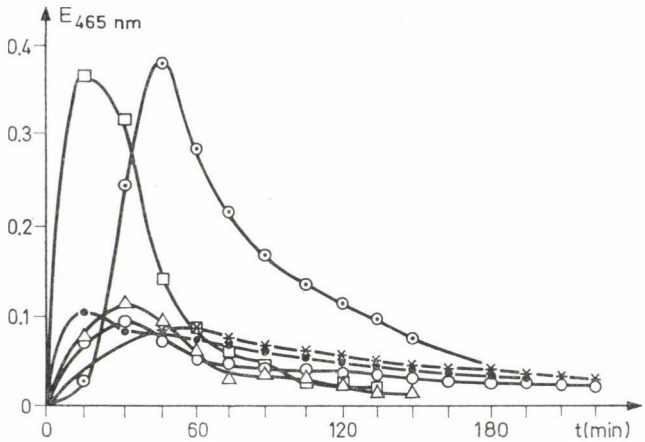

Fig. 4. Quantity of humic substances extracted by electroelution from meadow soil of Hosszúhát, as the function of the voltage applied and the time of electroelution, with Pierce cellophane filter and in citrate buffer (pH = 7.4). (+) 20 V, (○) 40 V, (.) 60 V, (X) 80 V, (●) 100 V, (□) 120 V

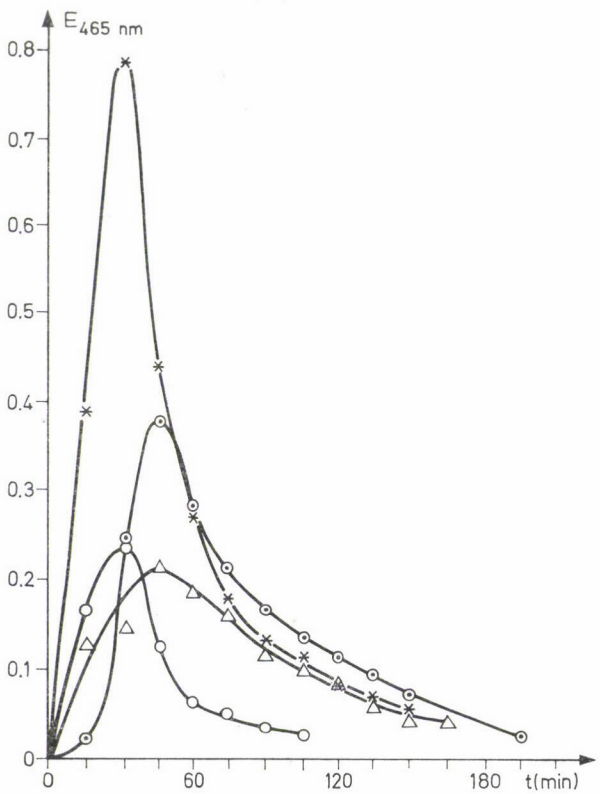

Fig. 5. Quantity of humic substances extracted by electroelution from meadow soil of Hosszúhát as a function of the time of elution, with various membrane filters used (pH = 7.4). (⊙) Pierce cellophane, (△) Kalle filter, (o) Vogel EUF filter, (+) double Whatman filter

The humic acid containing buffer solution flowing out from the separating tubes is collected in 10 cm^3 volumetric flasks, and stock solutions are prepared from then for further analyses.

Results

The examinations performed with the electroelution apparatus, combined with a membrane separation technique, are influenced by three parameters: the current used (its intensity, voltage and action period), the permeability of the membrane filter applied and the composition of the buffer. During the measurements described below the electroelution apparatus developed for research purposes was used.

In Fig. 4 the analytical results of the meadow soil of Hosszúhát, — a soil difficult to handle from the point of view of measuring technique — are given. The organic material content of the soil sample was 3.4%, and its clay content was 44.5%. The high clay content is unfavourable, because the membrane may be plugged in the course of measuring. Showing the measuring-results of the meadow soil of Hosszúhát, at the same time, we should like to prove that the procedure can even be used in case of soils of high clay content.

First the electric tension to be applied was determined. For this a citrate buffer of 7.4 pH was used (0.1 mol citric acid was dissolved in 400 cm^3 of water, the pH value of 7 was adjusted by 1 mole carbonate-free sodium hydroxide. The pH of the solution diluted with distilled water to 1000 cm^3 became 7.4). As membrane, the high, 5000-D molecular mass permeability Pierce dialysing membrane was used. The correlation between electric tension and quantity of separated organic matter was examined using 20, 40, 60, 80, 100 and 120 V voltages (Fig. 4). It was found that the quantity of the separated organic matter only became significant under the influence of 100 and 120 V. We chose 100 V as the voltage to be applied, since with 120 V cooling was no more able to counterbalance warming. As it can be seen in Fig 4 a 3-3,5-hour electroelution is sufficient to extract the humic substances.

After the electric voltage and the time of electroelution have been determined, the membrane filter is chosen. The membrane filter has the task of retaining the coarse soil particles displaced by the electric current, and fractioning the humic substances. In the course of measuring, the following membrane filters were tried out: the previously mentioned Pierce 5000-D cellophane filter, a dialysing membrane of about 5000-D molecular mass permeability produced by Kalle, Vogel's universal EUF-filter of about 20000 D molecular mass permeability and Whatman's highly permeable 1 μ pore-sized GF/C and GF/B glass "filter-paper" (Fig. 5). The quantity of the organic materials extracted is shown by the extinction measured at 465 nm wavelength (Lakatos et al. 1974). The calibration curve can be seen in Fig. 6.

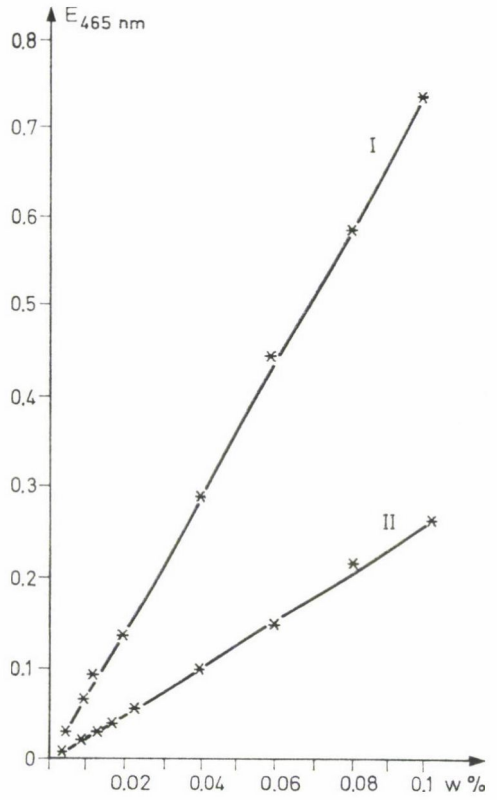

Fig. 6. Correlation between the concentration of humic substances and $E_{456\ nm}$, the extinction measured at 456 nm wavelength. (I) humic acid and (II) fulvic acid standard (Lakatos, B. et al. 1974). (w%) weight percentage of the aqueous solution

The Whatman filter showed low resistance to mechanical stress, so it had to be used in a double layer. The Whatman glass filter-paper retained only the clay particles. The Kalle membrane filter, on the other hand, lets through only the low molecular mass (below 5000 D) fulvic acids, and retains the other organic components (himatomelic acid, brown and grey humic acids). Thus, by the application of the two kinds of filter (Kalle and Whatman) the fulvic acid and humic acid contents of the soil can be simply, non-degradatively and quickly extracted and separated.

The task of the buffer used is to ensure the pH corresponding to the original condition of the soil in the course of measuring, that is, to compensate for the change of pH occurring during the electrophoresis.

During the measurements it was found that the citrate buffer used was unable to counterbalance the change of pH during the electroelution (pH = 1). Therefore, the higher buffer capacity citrate-$Na_4P_2O_7$ buffer was used (0.05

mole $Na_4P_2O_7$ and 0.05 mole citric acid were dissolved in 400 cm^3 of water, the pH value of the solution was adjusted to 7 with 1 mole sodium hydroxide solution, and it was diluted to 1000 cm^3. The pH value measured in the diluted solution was 7.5. When using such a buffer we did not find any measurable change of pH.

In order to improve the extractability of the humic substances carbamide was added to the citrate-$Na_4P_2O_7$ buffer, as carbamide has a strong desagyregating effect (0.05 mole citric acid, 0.05 mole $Na_4P_2O_7$ and 300 g carbamide were dissolved in 400 cm^3 distilled water, the pH of the solution was adjusted to 7 with 1 mole sodium hydroxide solution, and it was diluted with distilled water to 1000 cm^3, so the value of pH became 7.2. (Fig. 7) shows the quantity of fulvic- and humic acid, respectively, extracted from the meadow soil of Hosszúhát with the application of a citrate-sodium-pyrophosphate-carbamide buffer.

The clay content of the lime-coated chernozem soil of Nagyhörcsök was 23.1%, while its organic matter content determined by chromate method was 2.18%. With the electroelution technique at 100 V, in a citrate-sodium-pyrophosphate buffer and with the Whatman filter applied, 16 1% organic matter could be extracted from it in 160 minutes (Fig. 8). With the same voltage and buffer used, from the sandsoil of Őrbottyán nearly the total (0 91%)

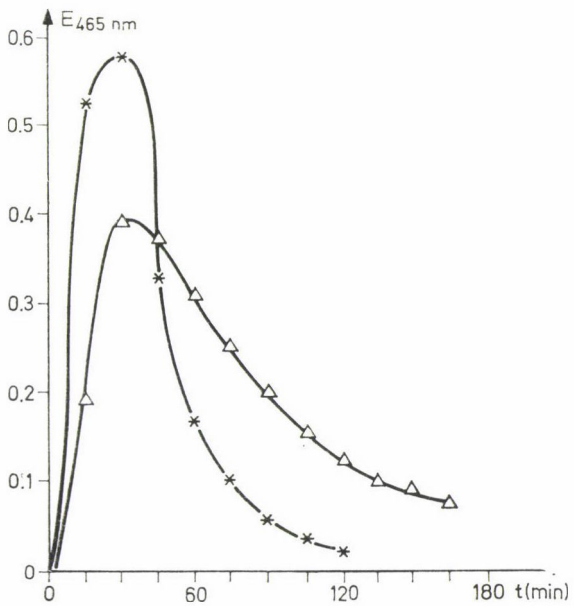

Fig. 7. Quantities of fulvic acid (Kalle filter) and humic acid + fulvic acid (Whatman filter) extracted by electroelution from meadow soil of Hosszúhát as the function of the time of electroelution, in citrate-sodium-pyrophosphate-carbamide buffer (pH = 7.2) at 100 V voltage. (+) double Whatman filter, (△) Kalle filter

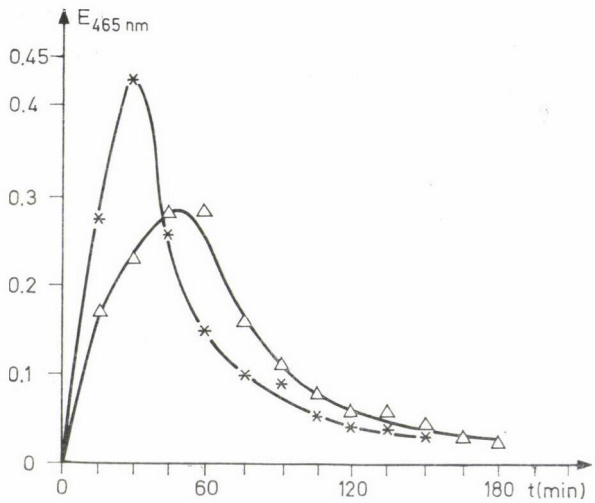

Fig. 8. Quantities of fulvic acid (Kalle filter) and humic acid + fulvic acid (double Whatman filter) extracted by electroelution from lime-coated chernozem soil of Nagyhörcsök as the function of the time of electroelution, in citrate-sodium-pyrophosphate buffer (pH = 7.2) at 100 V voltage. (+) double Whatman filter, (△) Kalle filter

amount of organic matter could be extracted in 75 minutes by electroelution (Fig. 9). Thus, the sandsoil of Őbrottyán has a low humus content, but this small quantity of organic material is very easily mobilizable.

With the method described, non-degraded fulvic acid and humic acid fractions can be extracted from the agricultural soils. When subjecting them to chemical-, analytical-, molar weight distribution- and structural (infrared absorption spectrometry, electron-spin resonance, magnetic resonance, mass spectrometry) examinations, a true picture may be obtained of the organic materials present in the soil. The shape of the electroelution curve provides data about the strength of clay mineral-organic matter bonds, and about the distribution of these organomineral bonds by strength.

With the routine electroelution apparatus 13, different samples, among them two "humus manures" were examined (Table 1).

As it by can seen from Table 1, with the electroelution method almost the same amount of organic matter can be extracted at pH 7 as with the highly efficient, though somewhat degrading sodium-pyrophosphate (method II) extraction at pH 10. The amount of organic matter extractable with the sodium-pyrophosphate-phosphoric acid buffer of pH 7 (method III) essentially increases under the influence of electric current in case of most of the samples, but it is nearly the same in the other cases, too. This can be well seen when the data of column (5) are compared to those of columns (7) and (8) in the table.

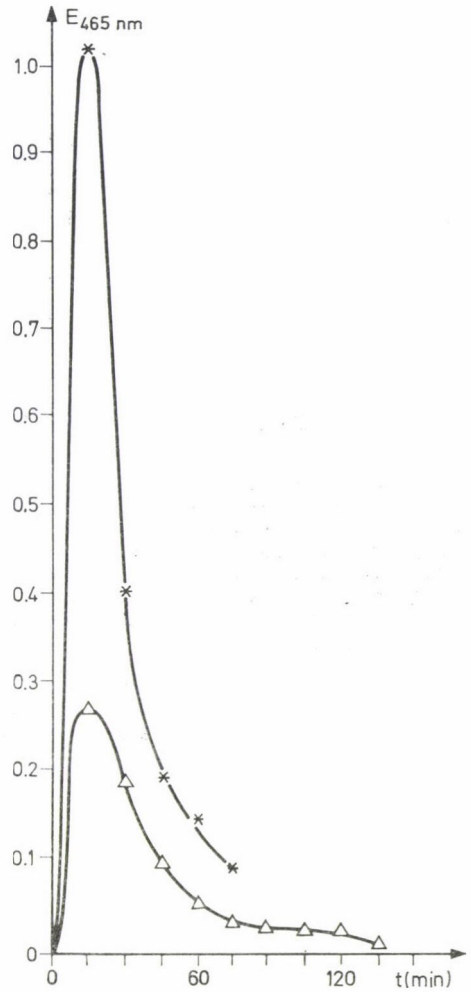

Fig. 9. Quantities of fulvic acid (Kalle filter) and humic + fulvic acid (double Whatman filter) extracted by electroelution from sandsoil of Őrbottyán as the function of the time of electroelution, in citrate-sodium-pyrophosphate buffer (pH = 7.4) at 100 V voltage. (+) double Whatman filter, (△) Kalle filter

By the comparison of the Q quotients it can be established that the fulvic acids could actually be separated, because when the Kalle filter was applied Q-values higher than 10 were obtained which are characteristic of the fulvic acids.

Besides being simple, the great advantage of the electroelution method over the extraction methods is that occasionally difficult filtering problems do not arise. Owing to filtering problems the fine clay particles often cannot be perfectly separated from the extracts. With the electroelution method, the

pure fractions having passed through the microfilters are obtained. The application of such fine filters would be practically impossible without electric current.

In the extraction methods the separation of fulvic acid from humic acids is carried out by acidification. By agreement we call fulvic acids the organic matter fraction which remains in aqueous solution even in acidic (pH = 1–2) medium. With the electroelution technique separation is really carried out by molecular mass at pH 7 by the help of filters, so it means a further improvement that the organic material is also in its natural condition during the whole process of separation. The more real humic acid-fulvic acid ratios thus obtained are shown in column (11) of Table 1.

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 31–38 (1991)

EFFECT OF PROLONGED FERMENTATION OF BIOGAS MANURES ON THEIR FERTILIZING EFFICIENCY

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(Received: 9th March 1988; accepted: 6th June 1988)

The influence of the fermentation period of biogas manure, derived from two sources, namely, mixture of maize stalks+cattle dung and cattle dung only, on the nutritional status of sandy soil and the growth of maize plants was studied.

Advancing the maturity of the biogas manures, 50 through 135 days, improved the nutritional properties of the soil. On the basis of plant growth, the 95-day fermentation period proved best to produce properly mature manure from both sources. Less mature and overmature manures were obtained from the 50- and 135-day digestion duration, respectively. Growth parameters of the 60-day old maize plants (dry weight and NPK contents of roots, shoots, and whole plants) showed their highest figures with the 95-day fermented mixture of maize stalks+cattle dug.

Keywords: biogas, biogas manures, fertilizing efficiency, maize, plant growth.

Introduction

The recent energy crisis and the resultant widespread fertilizer shortages, and their high prices, which make their use uneconomic for certain crops, have forced developing countries to review the problem in order to make the fullest use of indigenous resources. Hence, the possibility of using the biological process of anaerobic digestion to reconcile the conflicting needs of both fuel and manure from the same sources (cattle dung and crop wastes) has stimulated renewed worldwide interest in biogas plants. A biogas plant generates the combustible gas methane through the fermentation of organic wastes and refuse materials without impairing their manurial value (FAO 1977).

The role of manures in sustaining soil fertility has long been well established. Manures differ in their efficiency as fertilizers according to their origin and processing techniques. Manure obtained from a biogas plant is known to be rich in both macro- and micro-nutrients (Sathianathan 1975; Hornick et al. 1979; Abdel–Aziz et al. 1982). The Chinese scientists reported that biogas manures increased the crop yields by 17%. The application of effluent to wheat promoted tillering rates and the number of spikelets over those obtained from ammonium chloride (FAO 1977). Leo (1976) found that biogas manure increased the crops of maize, rice, cotton, and wheat by 28, 10, 24, 7, and 12.7% respectively as compared to the application of unfermented excreta.

Buren (1979) denoted that maize, rape vegetable, and wheat yields increased by 28, 25, and 16% respectively, when biogas manure was used. Alaa El-Din et al. (1984), in a comprehensive work, found that biogas manure surpassed the traditional fertilizers and the earth compost in increasing the yield of a number of field and horticultural crops.

The present work was undertaken to study the fertilizing efficiency of biogas manures fermented for varying periods, on the nutritional status of sandy soil and growth of maize plants.

Materials and methods

Maize stalks mixed with 30-day spent dung slurry, as well as fresh cattle dung, were used as raw organic residues. The spent dung slurry, added to the maize stalks, served as a bacterial starter (seeding material), and was derived from cattle dung diluted with water to bring the total solids content to 8–10%, then introduced into a household biogas digester (Sathianathan 1975) and left for 30 days.

Maize stalks were chopped into small pieces (3–5 cm), then mixed with the dung slurry and water to make a constant total solids content of 8%. The fresh cattle dung was diluted with water to bring the total solids content to 8%. These feedstocks were introduced into a Chinese-type biogas digester (Hobson et al. 1981). This digester with a fixed drum, had a capacity of 200-L digestion volume and 100-L unmovable gas space. The spent slurry represented 25% of the digestion volume, in the case of maize stalks. Batch fermentation techniques were followed at 28–30 °C.

Six digesters were allocated for each organic residue. Lime water was added at a rate of 2% at time to compensate for the expected drop in pH. Samples of the digested matter were collected from the fermentors at intervals of 50, 95, and 135 days, according to the rate of biogas production; namely, after each of the active, steady, and decline phases. Analytical data of such fermented residues are shown in Table 1 and the methods of analysis followed those Chapman and Pratt (1961).

Table 1

Elemental analysis of the biogas manures used as fertilizers

Manures applied	Fermentation period (days)	O.C* (%)	T.N** (%)	C/N ratio	Soluble N (ppm)	Soluble P (ppm)	Soluble K (ppm)
Fermented mixtures of:							
Maize stalks + Cattle dung	50	49.60	1.48	33.51	571	224	424
Maize stalks + Cattle dung	95	43.40	1.66	26.18	487	310	441
Maize stalks + Cattle dung	135	41.00	1.68	24.40	436	320	453
Fermented Cattle dung only	50	32.50	1.41	23.05	842	125	368
Fermented Cattle dung only	95	30.20	1.52	19.87	758	137	393
Fermented Cattle dung only	135	28.90	1.56	18.52	675	148	401

* O.C = Organic carbon.
** T.N = Total nitrogen.

A greenhouse experiment was carried out to evaluate the fertilizing efficiency of the organic residues fermented for varying periods. Sandy soil, collected from southern Tahreer Province (in Egypt) and having the properties shown in Table 2, was amended with the

digested organic residues at a rate of 40 kg N/feddan* (W/W) and placed in earthenware pots 5 kg each, in four replicates. The soil analysis was run according to Piper (1955). After two weeks of manure addition, the pots were planted with five maize kernels (*Zea mays*). Upon emergence, plants were thinned to three per pot. A second dose of the organic supplements was added, after one month of the first application (15 days after planting) at a rate of 20 kg N/feddan. Super phosphate was added, at planting time at a rate of 15 kg P_2O_5/feddan. The moisture content of the potted soil was maintained throughout at 60% of its water-holding capacity. The soil was analyzed for nutrient contents 60 and 75 days following the first manure application.

Some 60-day-old maize plants were dried at 75 °C and subjected to the determinations of dry weight and nutrient contents of the whole plants, divided into roots and shoots.

A nutrient analysis in soil and plants was performed according to the methods described by Chapman and Pratt (1961), as follows:

(a) Total nitrogen, by *Kjeldahl* method.
(b) Soluble nitrogen, by micro-steam distillation, in alkaline medium using Divarda alloy.
(c) Available phosphorus, colourimetrically, in the 0.5 M $NaHCO_3$ extract.
(d) Available potassium, flame-photometrically, in the ammonium acetate extract.

All figures reported herein are averages of duplicate determination expressed on a moisture-free basis.

Table 2

Physical and chemical properties of the sandy soil under consideration

Physical properties	Value	Chemical properties	Value
Particle fraction (%):		Organic matter (%)	0.36
Coarse sand	40.44	Total N (%)	0.09
Fine sand	40.91	Total P (%)	0.03
Silt	5.00	$CaCO_3$ (%)	4.51
Clay	9.33	pH (1 : 2.5)*	8.10
		E.C.** (m mohs/cm)	0.59
Texture grade	Sandy	Soluble ions (meq/100 g):	
		K^+	0.04
Water-holding capacity (%)	40.00	Na^+	1.37
		Ca^{2+}	1.00
		Mg^{2+}	0.50
		$H_2CO_3^-$	0.50
		Cl^-	0.25
		SO_4^{2-}	1.43

* Soil-water suspension.
** Electric conductivity.

Results and discussion

Nutrient status in soil

Changes of nutrient contents in the sandy soil, amended with the different biogas manures, are displayed in Table 3. Data presented for control treatment revealed decreases in the elemental analysis of the soil by advancing

* feddan = 4200 m^2 in area and 1×10^6 kg in weight of the depth 0–15 cm.

the determination period. This is, certainly, referred to the plant absorption, as well as to the loss in gaseous forms, particularly in the cases of carbon (as CO_2) and nitrogen (as NH_3).

The application of the biogas manures improved the nutritional properties of the soil. The second estimation duration showed a diminution in the

Table 3

Nutrient status of the sandy soil amended with different biogas manures, at two different doses making 60 kg N/feddan

Manures applied	Fermentation period (days)	Organic carbon (ppm)		Total nitrogen (ppm)		Available nitrogen (ppm)		Available phosphorus (ppm)		Available potassium (ppm)	
		A*	B**	A	B	A	B	A	B	A	B
Control (no addition)	—	1600	1352	588	401	6.0	2.0	10.6	5.6	99	84
Fermented mixture of:											
Maize stalks + Cattle dung	50	5120	4580	630	472	23.1	19.3	26.3	23.2	162	146
Maize stalks + Cattle dung	95	4980	4366	612	460	25.3	20.1	38.1	31.2	225	172
Maize stalks + Cattle dung	135	4862	4108	583	456	15.4	6.0	39.4	33.4	246	211
Fermented Cattle dung only	50	4090	3760	618	465	16.2	13.6	22.3	19.3	152	131
Fermented Cattle dung only	95	4010	3644	577	447	22.5	18.4	27.5	21.5	178	145
Fermented Cattle dung only	136	3912	3517	569	431	19.1	15.3	31.7	22.6	189	163

* A = 60 days after the first application of manure (40 kg N/feddan).
** B = 60 days after the second application of manure (20 kg N/feddan). Lapse between the two doses was 30 days.

contents of all nutrients examined. A prolongation of the fermentation interval for the digester stuffs resulted, generally, in reducing the contents of organic carbon and soluble nitrogen, but those of soluble phosphorus and potassium increased (Table 2). This is attributed to mineralization processes that took place throughout the fermentation course of the feedstock materials. Decreases in organic carbon and soluble nitrogen occur via evolution of CO_2 and probable volatilization of NH_3, respectively. An appreciable portion of NH_4^+-N is assimilated by many groups of the fermentative bacteria as a main source of their nitrogen (Bryant 1974; McLnerney and Bryant 1981). Contents of total N (%), shown in Table 1, were developed with fermentation time of the organic residues. This does not indicate any increase in the absolute total N amount, but in that relative to the organic matter as a whole. However, the extent of changes occurring within the interval 50–95 days of fermentation was higher than that within the last interval (95–135 days). The reason is that the digestion of the raw organic wastes leads to a vigorous production of both carbonaceous gases, CH_4 and CO_2 (composing biogas), within the primary stages of fermentation. As the process proceeds, the growth rate and activity of the

responsible bacterial agents decline as a result of by-product accumulation. Hence, the rate of nutrients liberation decreases.

The nutrients position that resulted from extending the digestion period of biomass was reflected in the enriched soil (Table 3). Otherwise, all of the measured elements showed decreases at the second detection time, due to the plant uptake and gaseous losses (of C and N particularly).

The fermented cattle dung possessed a narrower C/N ratio, a higher soluble N, and lower available P and K cotents than did the digested slurry of maize stalks + cattle dung mixture (Table 1). Such a situation was also reflected in the soil status (Table 3). Nevertheless, it should be considered that the narrow C/N ratio of the animal wastes encourages, when applied to the soil, the decomposition rate of both native and supplemented organic compounds, resulting in a rapid liberation of high amounts of the mineral constituents. If the amounts of such available nutrients exceed the needs of the growing plants, they will be subjected to loss by leaching and volatilization

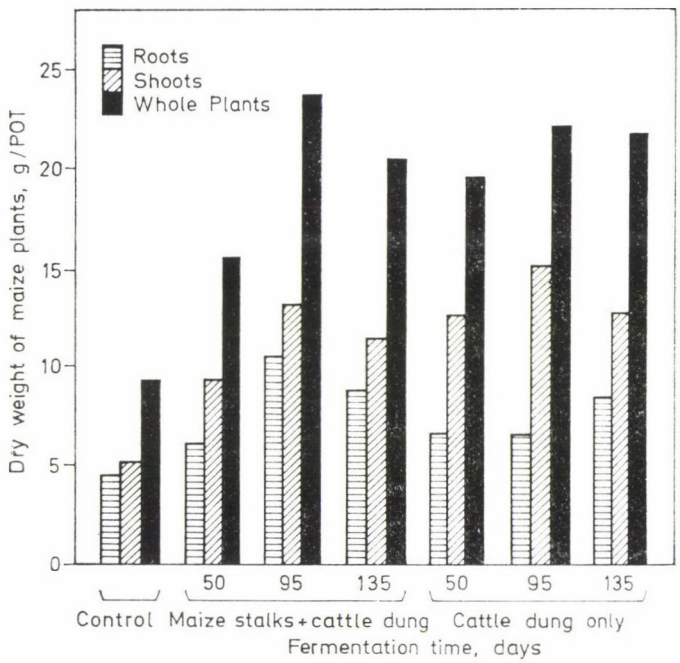

Fig. 1. Dry weight of maize plants (60 day old) grown in the sandy soil, as affected by different biogas manures fermented for varying periods and applied at a rate of 60 kg N/feddan

For instance, when ammonia is produced in great amounts, from N-rich substances, it leads to a temporary rise in soil pH, and a considerable quantity of it will be volatilized into the atmosphere. In either case, the biological system of the soil, i.e., microbial inhabitants and growing plants, will be

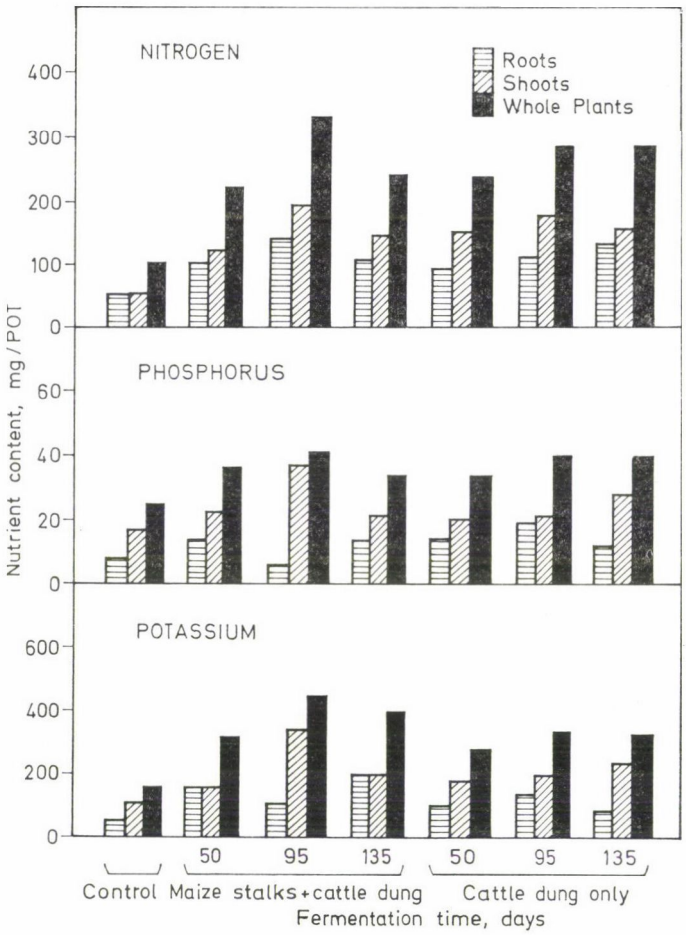

Fig. 2. NPK Contents in maize plants (60-day old) grown in the sandy soil amended with different biogas manures fermented for varying periods and applied at a rate of 60 kg N/feddan

injured. Furthermore, the hasty release of ammonium and its rapid oxidation to nitrate by the nitrifying community, results in the loosing of appreciable amounts of soil N by the leaching of such soluble unadsorbable forms of the element. These consequences are undesirable from the standpoints of both agronomy and public health (Alexander 1977).

Growth of maize plants

The dry weight of maize plants (60-day old) grown in the sandy soil amended with biogas manures is shown in Fig. 1. The organic additions, generally, enhanced the soil productivity as is shown by the augmented dry weights

of the whole maize plants. The 95-day fermented manure of either source tested revealed the top figures, whereas the 135-day 50-day old manures followed respectively. The uppermost value was recorded for the treatment with 95-day digested slurry of maize stalks + cattle dung mixtures. However, both 135- and 50-day fermented cattle dung gave higher results than did the corresponding ones of the mixture. Plant shoots showed higher weights than the roots, and both organs exhibited a similar trend to that observed for the whole plants in response to the organic additives used. Weight differences between shoots and roots in the treatments with the fermented mixture were narrower than those with the fermented dung only.

The effect of the application of differently fermented biogas manures to the sandy soil on the nutrients content of maize plants (60-day old) is illustrated in Fig. 2. The various manuring treatments showed the same trend for the three nutrients estimated in plant organs. Generally, the NPK contents were improved by the organic supplements, among which the 50-day fermented slurry of both sources gave the least values. NPK levels of the entire plants showed their greatest contenst from the 95-day fermented slurry. Shoots acquired higher nutrient contents than roots in all cases. The highest NPK uptake was obtained by the 95-day digested slurry of the maize stalks + cattle dung mixture. The 95-, and 135-day fermented cattle dung, and 135-day digested mixture followed respectively in action. Such trends accorded with that of the dry weight (Fig. 1). Maximum NPK contents of the shoots were observed by the treatment with 95-day digested slurry of the mixture, whereas those of the roots appeared with the 135-day of the same mixture.

These results indicated that the 95-day fermentation of biogas feedstocks proved to be the best period for manure output. Such mature manure is considered to be a balanced source of available nutrients, as well as a suitable supplier of plant growth regulating substances, as well as its assumed improvement of the physical characteristics of the soil. Likewise, the presence of appreciable amounts of those chelating compounds that promote the availability of polyvalent cationic nutrients is most probable at this stage. The 50- and 135-day digestion duration resulted in the production of immature and over-mature manures respectively. In the short period, adequate mineralization had not taken place; whilst in the long period, the highest mineralization level reduces the favourable action of the manure on the physical properties of soil, on one hand, and narrows the opportunity for the accumulation of growth regulators and chelating compounds, on the other.

The 95-day digested slurry of maize stalks + cattle dung mixture surpassed the corresponding manure deriving from the cattle dung only. This can be elucidated by the presence, in the first mixture, of higher amounts of plant growth-stimulating substances, such as auxins (IAA) and gibberellins (of plant origin), as well as of appreciable quantities of micronutrients. Moreover,

the high amounts of ammonia liberated from the rapid decomposition of the fermented animal manure (with narrower C/N ratio) might harm the emerging plant seedlings (Olsen and Kurtz 1982).

The comparative effect of biogas slurry and farmyard manure on wheat growth was studied by Zohdy and Badr El-Din (1983). They obtained greater yield and higher NPK contents with the biogas manure than with that from the farmyard. Similar results were found on corn by Imam et al. (1984): Sathianathan (1975) noted that fertilization with biogas manure makes supplemental application with nutrient elements unnecessary. He added that biogas manure serves a double purpose since it is both a soil conditioner and a fertilizer. Such manure, besides furnishing plant food, benefits the soil by increasing its water-holding capacity and improving its structure.

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 39–47 (1991)

Plant Physiology and Biochemistry

EFFECT OF TOXIC METALS INHIBITING THE GROWTH OF PLANT CALLUS TISSUES (IV.)

(Haploid callus tissues of *Nicotiana silvestris*)

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(Received: 24th April 1989; accepted: 10th July 1989)

The authors studied the effect of eight toxic metals contaminating our environment on the growth of haploid callus tissue cultures produced from *Nicotiana silvestris* anthers. The metals used were added to the sterile cell population in the form of the following compounds at concentrations between 10^{-4} and 10^{-7}: $Al_2(SO_4)$, CrO_3, $CuSO_4$ $5H_2O$, $HgCl_2$, $NiSO_4$, $Pb(NO_3)_2$, $Zn(SO_4)_3$ and Cd-acetate. The conditions of the experiments were the same as those described in the authors' earlier publications (Maróti and Bognár 1985, 1988, 1989).

As shown by the results of the experiments each of the eight metal compounds examined inhibits the matter increase of the callus tissues. The extent of inhibition ranged between 90,6% and 3,8% depending on the metals and concentrations used, as indicated by the figures of the tables (Tables 1–8). Another regular trend is that the fresh matter and the dry matter percentage change in opposite directions with the metal concentrations used. This fact is connected with the dehydration of cells, which is a criterion of the toxicity of metals. The experiments — with the earlier published results (Maróti and Bognár 1985, 1988, 1989) taken into consideration — stress the necessity of testing the effect of toxic metals on cultivated plant species as soon as possible, since their tolerance may essentially vary with species, and with genetic and physiological conditions.

Keywords: *Nicotiana silvestris*, haploid callus tissues, toxic metals

Introduction

Toxic metals have appeared in all spheres of our environment (air, water, soil) and both their number and concentrations increase constantly despite our attempts at prevention. For this reason their harmful effects must be increasingly understood in the future, especially if we wish to increase the organic matter production of plants. When doing so, we supposedly are forced to make use of organic material (e.g. sewage-sludge, or silting up causing the stagnation of waters) the toxic metal content of which is not as yet known. It would, therefore, be desirable to identify as soon as possible the toxic metals accumulating in the living organisms, first of all in the different organizational levels (cells, tissues, organs, intact plants) of various plant species and -varieties, and examine their effect on different objects included in experiments. Then from

the information thus obtained conclusions could be drawn on their effect manifested later in higher organisms (animals, humans).

In our experiments, motivated by the above goals we found that the same metal compound had highly different effects on the growth metabolism of isolated callus cultures from two plant genera (*Nicotiana tabacum* and *Ruta graveolens*), or with different concentrations of the same compound, different results were obtained as regards matter increase or cell number. These data confirm previous studies (Maróti and Bognár 1985, Kovács et al. 1986, Pajzs 1980, Turcsányi 1986).

In the work reported here, we studied the effects of several toxic metals on matter increase and cell number in a haploid callus culture of *Nicotiana silvestris*, another species of the genus *Nicotiana*.

Materials and methods

In our earlier experiments (Maróti and Bognár 1985, 1988) the effect of some contaminating heavy metals on the growth of callus cultures was studied in the plant species *Nicotiana tabacum* and *Ruta graveolens*.

In the present work the callus of another species of the genus *Nicotiana* was used for testing toxic metals. Our experimental material was a haploid cell population of *Nicotiana silvestris* produced from an anther culture five years ago. The cultures grew on a Murashige Skoog (1962) culture medium under identical physical conditions, as it was described earlier (Maróti and Bognár 1985, 1988, 1989). The growth parameters of the cultures were characteristic of the species, and the matter increase of the control cultures was, after 4 weeks, about 5 1/2 times that of the original inoculum.

The heavy metals to be tested were used in the experiments in the following forms and concentrations. 10^{-4}–10^{-6}M: $Al_2(SO_4)_3$, CrO_3, $CuSO_4 \cdot 5H_2O$, $NiSO_4 \cdot 7H_2O$, $Pb(NO_3)_2$, $ZnSO_4 \cdot 7H_2O$, Cd-acetate and 10^{-5}–10^{-7}M $HgCl_2$. The figures given for the variants are averages of 10 tissue samples (test-tubes) each, for which the scatter was also calculated. To investigate the effect of the compounds tested, we took the initial and final fresh matter and percentage dry matter content of the inocula, and counted the cells in the weight unit after Thomas and Davey (1975). From the data thus obtained we calculated the daily and the so-called relative growth rate for the cultures; the latter indicates the multiplication of the wight of the original inoculum. The data obtained by measuring and calculation were summarized in a tabular form, and also expressed as a percentage of the control (Reinert and Yeoman 1982).

Results and discussion

The numerical results of the experiments are contained in Table 1–8.

The experiment data obtained, both the absolute values (fresh and dry matter, change in the number of cells) and the converted values (daily and relative change, trend of percentile indices) are worth being considered as a function of the compounds used, on the one hand, and of an increase in their concentration, on the other.

A common characteristic of the eight metals examined as that each of them inhibited the matter increase of the isolated haploid callus tissues. The extent of inhibition ranged from 90,6% to 3,8%, depending on the kind and concentration of the metal used, and increased in each case with an increase

Table 1

Effect of aluminium on the growth of Nicotiana silvestris callus tissues

Hormones mg/l	$Al_2(SO_4)_3$ M		Fresh mass g/flask	Fresh mass % of control	Dry matter %	Dry matter % of control	Number of cells 10^3xn/g	Number of cells % of control	Daily mass increase mg	Daily mass increase % of control	Relative growth n x inoculum	Relative growth % of control
		$\bar{x}$	0.162		1.10		1140					
IAA	10^{-4}			12.1		101.8		66.0	—	—	—	—
2.0		±s	0.015		0.06		98					
		$\bar{x}$	0.149		1.00		920					
	$5 \cdot 10^{-5}$			11.1		92.6		53.3	—	—	—	—
		±s	0.020		0.07		61					
		$\bar{x}$	0.555		1.10		960					
Kin	10^{-5}			44.4		101.8		55.6	13.14	32.3	1.77	31.5
0.2		±s	0.184		0.07		51					
		$\bar{x}$	0.604		1.20		1060					
	$5 \cdot 10^{-6}$			45.1		111.1		61.4	14.96	36.8	2.02	36.0
		±s	0.148		0.09		55					
GA_3		$\bar{x}$	0.631		1.00		1110					
1.0	10^{-6}			47.1		92.6		64.3	15.96	39.2	2.15	38.3
		±s	0.118		0.08		32					
		$\bar{x}$	1.339		1.08		1727					
	Control			100.0		100.0		100.0	40.67	100.0	5.61	100.0
		±s	0.044		0.21		10					

Table 2

Effect of chromium on the growth of Nicotiana silvestris callus tissues

Hormones mg/l	CrO_3 M		Fresh mass g/flask	Fresh mass % of control	Dry matter %	Dry matter % of control	Number of cells 10^3xn/g	Number of cells % of control	Daily mass increase mg	Daily mass increase % of control	Relative growth nx inoculum	Relative growth %of control
		$\bar{x}$	0.140		0.99		1078					
	10^{-4}			10.4		91.7		62.4	—	—	—	—
IAA		±s	0.037		0.15		25					
2.0		$\bar{x}$	0.165		0.71		1207					
	$5 \cdot 10^{-5}$			12.3		67.5		69.9	—	—	—	—
		±s	0.052		0.07		23					
		$\bar{x}$	0.293		0.65		1202					
Kin	10^{-5}			21.9		60.2		69.6	3.32	8.2	0.46	8.2
0.2		±s	0.068		0.10		20					
		$\bar{x}$	0.355		0.67		1212					
	$5 \cdot 10^{-6}$			26.5		62.0		70.2	5.53	13.6	0.77	13.7
		±s	0.042		0.04		12					
GA_3		$\bar{x}$	0.498		0.52		1309					
1.0	10^{-6}			37.2		48.1		75.8	10.64	26.2	1.49	25.5
		±s	0.043		0.07		27					
		$\bar{x}$	1.339		1.08		1727					
	Control			100.0		100.0		100.0	40.67	100.0	5.61	100.0
		±s	0.044		0.21		10					

in the concentration. Fresh matter and dry matter content changed in opposite directions for almost all metals.

The increase in the fresh matter did not even reach 50% of the control at the lowest concentrations of Al, Cd, Cr, Cu, Hg and Ni, while Zn caused higher than 50% matter inhibition only at higher concentrations applied — 10^{-4} of Pb; 10^{-4}—5.10^{-5} M of Al, Cd, Cu, Ni; 10^{-5}—10^{-6} M of Hg — even caused death. It was only with Zn that neither cell destruction nor high rate loss of water were observed, even at its highest concentration. The percentage proportion of the dry matter of tissues was higher with any concentration of each metal than it was in the control. The only exception was Cr, for which the proportion was 52–8% lower than in the control. The opposite tendency change in percentage dry matter compared to the fresh matter increase is in the first place due to the loss of the water content of cells, as indicated in our earlier studies for diploid callus tissues of *Nicotiana tabacum* and *Ruta graveolens* (Maróti and Bognár 1985, 1988, 1989; Maróti et al. 1984). The loss of water caused by toxic metals can be regarded as one of the morphological effects of toxicity. At any concentration of the metal compounds tested the number of cells per unit matter (g) generally was more than 50% of the control.

Table 3

Effect of copper on the growth of Nicotiana silvestris callus tissues

Hormones mg/l	$CuSO_4$ M		Fresh mass g/flask	Fresh mass % of control	Dry matter %	Dry matter % of control	Number of cells 10^3xn/g	Number of cells % of control	Daily mass increase mg	Daily mass increase % of control	Relative growth n × inoculum	Relative growth % of control
		$\bar{x}$	0.170		1.16		1242					
	10^{-4}			12.7		107.4		71.9	—	—	—	—
IAA		±s	0.010		0.34		73					
2.0		$\bar{x}$	0.179		1.28		1143					
	$5 \cdot 10^{-5}$			13.4		118.5		66.2	—	—	—	—
		±s	0.010		0.11		42					
Kin		$\bar{x}$	0.385		1.31		1108					
0.2	10^{-5}			28.7		121.3		64.2	6.60	16.2	0.92	16.4
		±s	0.070		0.10		70					
		$\bar{x}$	0.652		1.45		1306					
	$5 \cdot 10^{-6}$			48.7		134.2		75.6	16.14	39.7	2.26	42.3
		±s	0.010		0.10		10					
		$\bar{x}$	0.707		1.10		1135					
GA_3	10^{-6}			52.8		101.9		65.7	18.10	44.5	2.53	45.1
1.0		±s	0.020		0.15		95					
		$\bar{x}$	1.339		1.08		1727					
	Control			100.0		100.0		100.0	40.67	100.0	5.61	100.0
		±s	0.044		0.21		10					

Table 4

Effect of nickel on the growth of Nicotiana silvestris callus tissues

Hormones mg/l	$NiSO_4$ M		Fresh mass g/flask	Fresh mass % of control	Dry matter %	Dry matter % of control	Number of cells $10^3 \times n/g$	Number of cells % of control	Daily mass increase mg	Daily mass increase % of control	Relative growth n × inoculum	Relative growth % of control
		$\bar{x}$	0.162		1.33		1299					
IAA	10^{-4}			12.1		123.1		75.2	—	—	—	—
2.0		$\pm s$	0.017		0.08		71					
		$\bar{x}$	0.179		1.61		1225					
Kin	$5 \cdot 10^{-5}$			13.4		149.0		70.9	—	—	—	—
2.0		$\pm s$	0.036		0.07		69					
		$\bar{x}$	0.820		1.17		1199					
GA_3	10^{-5}	$\pm s$	0.218	61.2	0.11	108.3	46	69.4	22.14	54.4	3.10	55.2
1.0		$\bar{x}$	0.907		1.17		1139					
	$5 \cdot 10^{-6}$			67.7		108.3		65.9	25.25	62.0	3.53	62.9
		$\pm s$	0.101		0.08		76					
		$\bar{x}$	0.957		1.15		1244					
	10^{-6}			71.5		106.5		72.0	27.03	66.5	3.53	62.9
		$\pm s$	0.261		0.03		55					
		$\bar{x}$	1.339		1.08		1727					
	Control			100.0		100.0		100.0	40.67	100.0	5.61	100.0
		$\pm s$	0.044		0,21		10					

Table 5

Effect of lead on the growth of Nicotiana silvestris callus tissues

Hormones mg/l	$Pb(NO_3)_2$ M		Fresh mass g/flask	Fresh mass % of control	Dry matter %	Dry matter % of control	Number of cells $10^3 \times n/g$	Number of cells % of control	Daily mass increase mg	Daily mass increase % of control	Relative growth n × inoculum	Relative growth % of control
		$\bar{x}$	0.186		1.44		1165					
	10^{-4}			13.9		133.3		67.5	—	—	—	—
IAA		$\pm s$	0.023		0.14		4					
2.0		$\bar{x}$	0.764		1.18		1197					
	$5 \cdot 10^{-5}$			57.0		109.2		69.3	20.88	51.3	2.82	50.3
		$\pm s$	0.264		0.13		21					
Kin		$\bar{x}$	0.710		1.14		1185					
0.2	10^{-5}			53.0		105.0		68.6	18.88	46.4	2.55	45.5
		$\pm s$	0.198		0.31		8					
		$\bar{x}$	0.601		1.36		1205					
GA_3	$5 \cdot 10^{-6}$			44.9		125.9		69.5	14.85	36.5	2.00	35.7
1.0		$\pm s$	0.211		0.09		7					
		$\bar{x}$	0.616		1.38		1194					
	10^{-6}			46.0		127.8		69.1	15.40	37.9	2.08	37.0
		$\pm s$	0.175		0.17		7					
		$\bar{x}$	1.339		1.08		1727					
	Control			100.0		100.0		100.0	40.67	100.0	5.61	100.0
		$\pm s$	0.044		0.21		10					

However, in the case of Al, Cr, Cu, Ni, Pb and Zn it did not reach the full value of the control, while in response to Cd and Hg the number of cells per unit matter was larger than in the control.

Table 6

Effect of zinc on the growth of Nicotiana silvestris callus tissues

Hormones mg/l	$ZnSO_4$ M		Fresh mass g/flask	% of control	Dry matter %	% of control	Number of cells 10^3x n/g	% of control	Daily mass increase mg	% of control	Relative growth n x inoculum	% of control
IAA 2.0	10^{-4}	$\bar{x}$	0.456	34.0	1.89	175.0	1175	68.0	9.14	22.5	1.28	22.8
		$\pm s$	0.067		0.25		341					
	$5 \cdot 10^{-5}$	$\bar{x}$	0.474	35.0	1.93	178.7	1400	81.0	9.78	24.0	1.37	24.4
		$\pm s$	0.149		0.23		75					
Kin 0.2	10^{-5}	$\bar{x}$	0.934	69.7	1.37	126.8	1317	76.3	26.21	64.4	3.67	65.4
		$\pm s$	0.318		0.17		73					
GA_3 1.0	$5 \cdot 10^{-6}$	$\bar{x}$	0.814	60.8	1.39	128.7	1580	91.5	21.92	53.9	3.07	54.7
		$\pm s$	0.096		0.27		122					
	10^{-6}	$\bar{x}$	0.981	73.2	0.95	88.0	1720	90.6	27.99	68.6	3.90	69.5
		$\pm s$	0.285		0.21		79					
	Control	$\bar{x}$	1.339	100.0	1.08	100.0	1727	100.0	40.67	100.0	5.61	100.0
		$\pm s$	0.044		0,21		10					

Our data unequivocally show that the metals examined act on the plants through the growth metabolism of the cells and tissues (growth inhibition, loss of water, mortality) and thereby, may affect the chain of nutrition. As known from literary data the metal compounds examined have caused inhibition within the mentioned limits of concentration in species other than those of the genus *Nicotiana*. For example, certain concentrations of Cd, Hg, Zn have a lethal effect on green alga cells (Philippis et al. 1981). According to Wallace (1977), Dijkshoorn et al. (1979) various amounts of Cd, Cu, Ni, Pb, Zn generally had a toxic effect on plant growth. But not only directly measurable morphological effects appearing in growth inhibition were observed in metal treatments. Inhibition of cell division and -motion was experienced in *Euglena* in response to Cd, Hg and Zn (Filippis et al. 1981). Furthermore, inhibition of the activity of a number of enzymes, and of the DNA synthesis of nuclei, as a result of Cd,

Table 7

Effect of cadmium on the growth of Nicotiana silvestris callus tissues

Hormones mg/l	$Cd(CH_3COO)$ M		Fresh mass g/flask	% of control	Dry matter %	% of control	Number of cells $10^3 \times n/g$	% of control	Daily mass increase mg	% of control	Relative growth n× inoculum	% of control
IAA 2.0	10^{-4}	$\bar{x}$	0.142	10.6	1.75	162.0	1040	60.2	—	—	—	—
		±s	0.021		0.14		23					
	$5 \cdot 10^{-5}$	$\bar{x}$	0.231	17.2	1.93	178.7	1080	62.5	—	—	—	—
		±s	0.010		0.17		22					
Kin 0.2	10^{-5}	$\bar{x}$	0.659	49.2	1.30	120.3	1770	102.5	16.39	40.3	2.29	40.8
		±s	0.210		0.27		11					
	$5 \cdot 10^{-6}$	$\bar{x}$	0.573	47.8	1.50	138.9	2090	121.0	13,32	32.8	1.86	33.1
		±s	0.150		0.33		19					
	10^{-6}	$\bar{x}$	0.648	48.4	1.90	175.9	1890	109.4	16.00	39.3	2.24	39.9
		±s	0.150		0.21		13					
GA_3 1.0	Control	$\bar{x}$	1.339	100.0	1.08	100.0	1727	100.0	40.67	100.0	5.61	100.0
		±s	0.044		0.21		10					

Cu, Hg, Zn applied was demonstrated (Bonaly et al. 1980, Weigel and Jäger 1980). Nag et al. (1981) even observed a negative effect exercised by some metals (Cu, Hg, Zn) on chlorophyll formation and photosynthetic activity in germinating rice plants.

From literary data and the results of our own experiments we can establish that some metal traces occurring in our environment may affect various plant species in different measures depending on their concentration. These effects mostly appear in metabolic inhibition. The danger increases when sludges imposing a harmful load on the environment are brought into contact with plants and animals, and finally with humans as receivers. It is therefore, now an urgent task to investigate and prevent the effect of toxic concentrations of various metals on plant species and ultimately on humans, since as our results show, not only the heavy metals but e.g. Al too may cause weight increase in the tissues of plant species.

Acknowledgement

The authors are indebted to Miss. Erzsébet Léh, assistant, for her useful technical collaboration.

Table 8

Effect of mercury on the growth of Nicotiana silvestris callus tissues

Hormones mg/l	$HgCl_2$ M		Fresh mass g/flask	Fresh mass % of control	Dry matter %	Dry matter % of control	Number of cells $10^3 \times$ n/g	Number of cells % of control	Daily mass increase mg	Daily mass increase % of control	Relative growth n x inoculum	Relative growth % of control
	10^{-5}	$\bar{x}$	0.126	9.4	1.61	149.0	1605	92.9	—	—	—	—
		$\pm s$	0.019		0.08		11					
IAA 2.0	$5 \cdot 10^{-6}$	$\bar{x}$	0.140	10.4	1.78	164.8	1545	89.5	—	—	—	—
		$\pm s$	0.015		0.15		13					
	10^{-6}	$\bar{x}$	0.154	11.5	1.90	175.9	2010	116.4	—	—	—	—
Kin 0.2		$\pm s$	0.013		0.22		39					
	$5 \cdot 10^{-7}$	$\bar{x}$	1.190	88.8	1.10	101.8	2050	118.7	35.35	86.9	4.95	88.2
		$\pm s$	0.056		0.13		11					
GA_3 1.0	10^{-7}	$\bar{x}$	1.289	96.2	1.00	92.6	1521	88.1	38.89	95.6	5.44	96.9
		$\pm s$	0.038		0.10		10					
	Control	$\bar{x}$	1.339	100.0	1.08	100.0	1727	100.0	40.67	100.0	5.61	100.0
		$\pm s$	0.044		0.21		10					

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 49–55 (1991)

INFLUENCE OF SHADING ON PRODUCTION OF CUCUMBER IN THE LATE SUMMER SEASON IN EGYPT

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(Received: 4th September 1989; accepted: 15th December 1989)

Experiments were carried out on cucumber — cv. Biet Alpha — during the late summer seasons of 1982 and 1983 in the Experimental Farm of Faculty of Agriculture, Kafr El-Sheikh, Egypt.

The main objective of this research was to study the effect of shade on cucumber production. The shade levels included 40, 55, 63% shade and unshaded control. Plot area was 31.5 m^2. Sowing date was May, 28, and shade nets were spread on solid iron framed tunnels (7.5 m wide and 2.75 m high) in June, 29, in both years.

The shade significantly decreased the production of staminate flowers and hastened the pistillate flowering.

The number of fruits/plant, total fruit yield and the yield of grade "A" increased as shading decreased.

Best results were obtained from 40% shade treatment.

Keywords: cucumber, vegetative growth, fruit yield, effect of shading

Introduction

The average yield of the cucumber crop at Kafr El-Sheikh Governorate in summer seasons of 1982 and 1983 was 18–23 ton/ha, which is rather low production and needs improvement.

It is known that the increase in light intensity and day length increase the number of staminate flowers (Cantliffe 1974, 1981). Kaname and Itagi (1970) on cucumber reported that shading by black cloth in winter, early spring and autumn, lowered the production of pistillate flowers.

On pepper, Quagliotti (1976) reported that shading doubled yields. However, heavier shading 50% shade reduced fruit yields. Jeon and Chung (1982), on red pepper, reported that fruit yield decreased as shading increased.

On the other hand, El-Aidy et al. (1983), under the same conditions of this study reported that the low shade level (40%) produced the highest fruit yield of tomato and improved the quality of fruits.

Many reports indicated that shading increased chlorophyll content (Guers 1974, Boardman 1977, Collard et al. 1977, Moon and Pyo 1981, Kappel and Flore 1983, El-Aidy et al. 1983).

The objective of this work was to evaluate the effect of some shade levels on cucumber production during the late summer season.

Materials and methods

Experiments were conducted in the Experimental Farm of Faculty of Agriculture, Kafr El-Sheikh, Egypt, during the summer seasons of 1982 and 1983. The variety used was Biet Alpha, widely grown and well-known in Egypt.

Sowing dates were on May, 28, in both years in double rows $100+50\times30$ cm. Plot size was 31.5 m^2. Four treatments were used. Shade materials (40, 55 and 63% shade grade) from Polypropolin were provided by Tildent Co. from England. Shade nets were spread on solid iron framed tunnels (7.5 m wide and 2.75 m high) on 29th June in both seasons.

The cultural practices and pest control were done as required.

The four shade levels were randomly distributed in four replications. Data were tested by analysis of variance. Duncan's multiple range test was used for comparisons among the means of treatment (Duncan 1965).

Results and discussion

Effect of shading

(1) Soil and air temperature

Data presented in Fig. 1 indicate that shading resulted in the reduction of soil and air temperature. The effect was more noticeable with soil temperature than air temperature.

It is expected that plant temperature would show a more noticeable response to the shade than that of air temperature. Leopold and Kriedemann (1975), reported that shade can reduce leaf temperature from 40 or 45 C in horizontally exposed foliage to about 30 to 32 C, that is, close to ambient air temperature.

(2) Vegetative growth

Data presented in Table 1 show that the plants grown under the shade had longer stems than those in the open. These results may be explained as a result of the effect of the shade treatments which altered the quality of incident light. This in turn might effect hormonal balance which could alter plant height. Moreover, our observations are in agreement with the findings of many researchers, Gray and Steckel (1981) on lettuce and El-Aidy et al. (1983) on tomato.

Data presented in Table 1 show that significant differences had been obtained in leaf area in both seasons. The control had the lowest record, while the highest leaf area was obtained from the 55% shade treatment in both seasons. It did not statistically differ from that of 63% shade treatment in the first season. The promotive effect of shade might be due to the increase in the number of leaves per plant which might lead to a similar increase in leaf area. On the other hand, shading increases leaf surface.

Table 1

Effect of shading on vegetative growth of cucumber at different stages (1982 and 1983)

Shading grade (%)	Stem length (cm)			No. of leaves/plant			No. of branches/plant	Leaf area/plant (cm^2)	Dry wt. of 5th leaf (gm)*
	Days after sowing								
	30	45	60	30	45	60	60	45	45
				First season 1982					
Control	45.0a	98.1b	154.4b	10.0a	21.9c	32.7c	11.6a	2813.8c	0.62a
40%	43.5a	119.0a	188.4a	9.0a	25.4b	44.5b	9.2b	6062.9b	0.55b
55%	44.1a	127.1a	203.2a	9.9a	27.5a	52.0a	6.6c	7652.8a	0.52b
63%	45.2a	125.7a	198.9a	9.8a	27.8a	51.6a	5.3d	7551.5a	0.48c
				Second season 1983					
Control	31.6a	125.5b	144.3c	9.1a	19.1c	30.8c	10.3a	2862.1d	0.60a
40%	31.1a	146.8ab	185.6b	9.0a	22.5b	39.3b	7.8b	5051.6c	0.53b
55%	30.9a	150.4a	197.3ab	9.0a	22.5b	46.2a	5.7c	6405.9a	0.51b
63%	30.2a	156.1a	207.7a	9.0a	25.1a	46.4a	5.4c	6175.1b	0.47c

* Dry weight of the fifth leaf from the growing tip of the plant.
Means designated by the same letter are not significantly different at the 5% level, according to Duncan's multiple range test.

(3) Flowering

Data in Table 2 show that shading decreased significantly the production of staminate flowers. The control produced the highest number of staminate flowers. However, there was no significant difference between 55% and 63%

Table 2

Effect of shading on flowering of cucumber plants (1982 and 1983)

Shading grade (%)	No. of flowers/plant		♂/♀ ratio
	Staminate	Pistillate	
	First season 1982		
Control	146.4a	11.7c	12.7a
40%	135.3b	15.3a	8.9b
55%	120.1c	14.4ab	8.4b
63%	120.2c	13.8bc	8.8b
	Second season 1983		
Control	141.2a	11.3b	12.7a
40%	132.5b	14.1a	9.5b
55%	119.9c	13.1ab	9.1b
63%	118.7c	13.1ab	9.3b

Means designated by the same letter are not significantly different at the 5% level, according to Duncan's multiple range test.

shade treatments in both seasons. This may be due to the effect of shading which reduced the light intensity and air and soil temperatures. These observations agree with those obtained by Cantliffe (1974 and 1981) on cucumber, which indicated that more staminate flowers were produced under high temperatures and high light intensity.

Results in Table 2 show a significant effect of shading on pistillate flowers. The low shade treatment (40%) produced the highest number of pistillate flowers in both seasons, while the control produced the least one. Early work (Kanama and Itagi 1970, Cantliffe 1981) showed that shading lowered the proportion of pistillate flowers of cucumber.

The staminate/pistillate ratio tended to decrease as shading grade increased, these results agree with those of Kanama and Itagi (1970) and Cantiffe (1974).

(4) Fruit yield

Data presented in Table 3 show that shading had a significant effect on the number of fruits per plant in both seasons. The 40% shade treatment produced the highest number of fruits per plant in both seasons, while the control had the lowest one. These results are due to the high number of pistillate flowers with 40% shading. Also, the reduction of air temperature might cause some improvement in fruit set, leading to higher fruit production under shade during summer season.

The 40% shade treatment produced the highest yield in both seasons, while the control had the least one. These results might be due to the high

Table 3

Effect of shading on fruit yield of cucumber (1982 and 1983)

Shading grade (%)	No. of fruits per plant	Yield (kg/m²)	
		Total	Grade "A"
	First season 1982		
Control	9.4c	3.5c	2.3b
40	13.9a	5.2a	4.6a
55	12.9ab	4.8ab	4.5a
63	11.8b	4.4b	4.1a
	Second season 1983		
Control	8.3d	3.1c	2.1c
40	12.5a	4.7a	4.1a
55	11.1bc	4.2b	3.8ab
63	10.6c	3.9b	3.6b

Means designated by the same letter are not significantly different at the 5% level, according to Duncan's multiple range test.

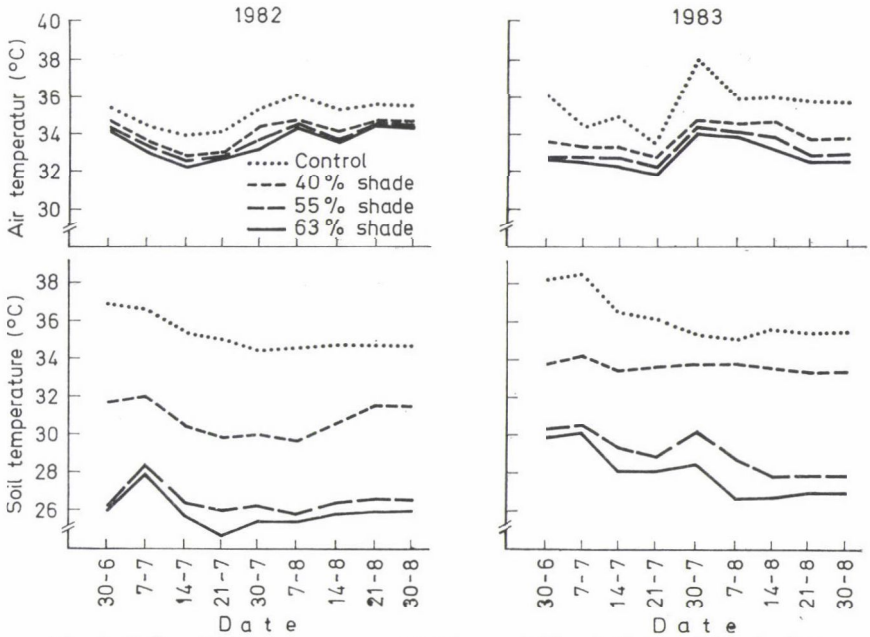

Fig. 1. Soil and air temperature at 2.00 p.m. (°C) as influenced by shading

number of fruits per plant produced under shade compared to the control. Many workers came to the same result indicating that the low shading (30–40%) almost doubled yields and improvement was due to the increase in fruit number (Quagliotti 1976 on papper and El-Aidy et al. 1983 on tomato).

(5) Fruit quality

Data presented in Table 4 show a significant effect of shading on fruit length. The fruit length increased as the shading grade increased. The control plants had the shortest fruits in both seasons, while the 63% shade treatment had the longest fruits, but it did not differ significantly from 55% shade treatment. This might be due to the effect of shading which increases cell division and expansion.

Also, there was a significant effect of shading on fruit diameter in both seasons. The control had the biggest diameter. The fruit diameter decreased as the shading grade increased. This may be explained as a result of cell expansion which increased under shading.

The highest L/D ratio was obtained from 63% shade treatment in both seasons.

(6) Total soluble solids (T.S.S.)

Data presented in Table 4 show that there was a significant effect of shading on T.S.S. in both seasons. T.S.S. decreased as the shading grade increased.

Table 4

Effect of shading on fruit quality of cucumber (1982 and 1983)

Shading grade (%)	Fruit length (cm)	Fruit diameter (cm)	Fruit L/D ratio	T.S.S. (%)
		First season 1982		
Control	13.94c	3.67a	3.79	4.55a
40	15.42b	3.57b	4.31	4.34a
55	16.13a	3.38c	4.77	3.30bc
63	16.19a	3.34c	4.84	2.97c
		Second season 1983		
Control	12.76c	3.73a	3.42	4.32a
40	14.36b	3.60b	3.92	3.37b
55	15.25a	3.40c	4.48	3.19b
63	15.68a	3.35c	4.68	2.70c

Means designated by the same letter are not significantly different at the 5% level, according to Duncan's multiple range test.

The control had the highest T.S.S., though it did not statistically differ from 40% shade treatment in the first season. El-Aidy et al. 1983, on tomato, obtained similar results showing that shading decreased the total soluble solids of fruits.

The highest yield of grade "A" in both seasons was obtained from 40% shade.

Table 5

Economic evaluation of shade nets for cucumber production in the summer season (P.T.+/m²) (1982 and 1983)

Treatments	Cost/m²		Annual depression/m²			1982		1983	
	Shade nets	Iron tunnels	Shade nets	Iron tunnels	Total	*Crop value/ m²	Net income (P.T.)*	Crop value/ m²	Net income (P.T.)*
Control	—	120	—	6.0	6.0	87.5	81.5	93.0	87.0
40% shade	88.0	120	8.8	6.0	14.8	130.0	115.2	141.0	126.2
55% shade	96.0	120	9.6	6.0	15.6	120.0	104.4	126.0	110.4
63% shade	104.0	120	10.4	6.0	16.4	110.0	93.6	117.0	100.6

* Crop value was estimated on the basis of sale price during the production period.
+ U $ = 2.6 L.E. and 1 L.E. = 100 P.T.

Economic evaluation

To evaluate the use of shade nets for cucumber production in the summer season, under Kafr El-Sheikh conditions, an estimation of the costs was done

Such evaluation included the costs of materials used (shade nets and iron structure of the tunnels).

The cost of shade nets was calculated on the basis of ten years as supposed by the production Co. (The materials used under Kafr El-Sheikh conditions from 1981 till now (1989) are still in good condition. The cost of iron structure of the tunnels was calculated on the basis of twenty years (this structure has been used in Kafr El-Sheikh from 1974 till now). The estimation of the crop value was done on the basis of sale price during the production period.

Data in Table 5 show that the cost increased steadily as the shading grade increased and the control had the lowest cost in both years.

The highest net income was obtained from 40% shade grade treatment, while the control gave the lowest net income in both seasons.

Thus, it can be concluded that 40% shading is the most effective shade for the best production of cucumber in the late summer season under Kafr El-Sheikh conditions.

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 57–67 (1991)

EFFECT OF GAMMA RADIATION AND TEMPERATURE ON POTATOES DURING STORAGE

II. Physiological and biochemical changes[1]

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(Received: 19th June 1989; accepted: 7th December 1989)

Potato varieties Desire and Metal were irradiated with 0, 50, 100 and 500 Gy gamma rays, and were stored at 5 °C and 20–25 °C for 6 months. Respiratory rate, starch, total and reducing sugar contents were determined every month. The control samples of *variety Desire* showed higher respiration rates in comparison with the irradiated tubers. The respiratory rate was significantly higher in the *variety Metal* in comparison with the *variety Desire*. The sugar content was higher in the irradiated tubers, and it was also higher in the tubers stored at 5 °C than in those stored at 20–25 °C. This increase in sugars accompanied with a decrease in starch content, suggests conversion of starch into sugars.

Keywords: gamma radiation, potato respiration, starch, storage, sugar

Materials and methods

Two types of potato varieties used in the present investigations, Desire and Metal the potatoes were produced in the state farm of Szentlőrinc (in the south of Hungary). Radiation treatment of potato was carried out by gamma irradiation from Cobalt-60 source at the pilot plant of AGROSTER Irradiation Company, Budapest. The radiation doses applied for both varieties were 50 Gy, 100 Gy and 500 Gy. Irradiated and control potatoes were collected in cases, by 20 kg per treatment and stored at 5 °C, also the same amount from each treatment for both varieties were stored at 20–25 °C. Texture investigation, sprouting and weight loss were measured regularly every month from December 1987 to June 1988.

Measurement of respiration

The respiration measurements were carried out using an Infra-red gas analyser (Type Infralyt 4, made in GDR). The flow rate of outside air and the amount of outside CO_2 were measured. About 1 kg of potato was weighed from each treatment for both varieties. A sample was placed in a thermostat at a temperature of 25 °C. A flow of air from outside passed through the potato sample and the CO_2 concentration was measured. The results were expressed in CO_2 mg/kg/h.

Total sugar determination

The sugar content of the potatoes was measured by a colorimetric method, using the phenol-sulfuric acid reaction according to Dubois et al. (1956). An approximate 1 g sample from each treatment was weighed and blended with boiling distilled water, then washed three

[1] Part I. see in Acta Agr. Hung. Vol 39, Numb. 3–4. pp. 331–340.

times. The homogenate was shaked for 20 min., then left for 10 min. It was centrifuged at 6000/min for 10 min. The supernatant was transferred to a 50 ml volumetric flask, which was filled up to capacity with distilled water. From the solution, 1 ml was transferred to a test tube, then 1 ml of 5% phenol, and 4 ml of concentrated sulfuric acid (98%) were added. After 10 min., the absorbance of the mixture was measured at 485 nm by spectrophotometer. The concentration of total sugars (μg/mg) was calculated using a calibration curve of D-glucose.

$$\text{Total sugar } (\mu\text{g/mg}) = \frac{50 \times \text{concentration of glucose } (\mu\text{g/ml})}{\text{sample weight in mg}}$$

Three replicates for each treatment for each storage temperature of each potato variety were carried out.

Reducing sugar determination

Reducing sugar content of potatoes was measured by a colorimetric method using the Anthron reaction. (Witham et al. 1971). From each treatment, 1 g of both varieties was blended with 3 ml of 96% ethanol, then washed three times with 3 ml of 80% ethanol. The homogenate was filtered and the filterate transferred to a 20 ml volumetric flask, which was filled up to capacity with 80% ethanol. Then 1 ml of the solution was transferred to a test tube, to which 4 ml of Anthron solution were added, shaken, and placed in a 50 °C hot water for 10 min. Afterwards the absorbance of the mixture was measured at a 620 nm by spectrophotometer. The content of reducing sugars (mg/g) was calculated using a calibration curve of D-glucose.

$$\text{Reducing sugars (mg/g)} = \frac{20 \times \text{concentration of glucose (mg/ml)}}{\text{sample weight in grams}}$$

Three replicates for each treatment and for each storage temperature of each potato variety were analysed.

Starch determination

The starch content determination was carried out according to the method of McCready et al. (1950). After the extraction of sugars the residue was used for starch analysis; 30 ml of 35% perchloric acid was added and mixed well. The mixture was shaken for 20 min., then left for 10 min., after which it was transferred to a centrifuge tube, and centrifuged at 6000/min. for 10 min. The supernatant was separated and transferred to a 100 ml volumetric flask, the remainder mixed with 15 ml 35% perchloric acid, shaken and centrifuged, then the supernatant was transferred to the volumetric flask, which filled up to capacity with distilled water. From the solution, 1 ml was transferred to a test tube, to which 1 ml (5%) phenol, and 4 ml of concentrated sulfuric acid (98%) were added. After 10 min., the absorbance was measured at 485 nm by a spectrophotometer, and the starch content (μg/mg) was calculated using calibration curve of D-glucose.

Results and discussion

Respiration results

The respiration rate in the Desire tubers stored at 5 °C decreased in the first 2 months of storage period, then it increased or slightly varied. The unirradiated tubers showed a higher respiration level (21.5 mg CO_2/kg/h) than the irradiated ones, and the irradiated tubers showed a decrease in the respiration (3.6, 3.7 and 5.8 mg CO_2/kg/h in 50, 100 and 500 Gy treatments, respectively) during the 6 months of storage, which might be due to the effect of

irradiation on the enzymes. In the *variety*, *Desire* tubers stored at 20–25 °C, the respiratory rate decreased also in the first 2 months of storage, then it increased to the maximum in the 5th month in the control tubers (37.9 mg CO_2/kg/h) accompanied with a decrease in sugar content, but in the irradiated tubers the respiration rate was maintained almost at the same level (between 3.8 and 10 mg CO_2/kg/h) at the 2nd month of the storage period (Fig. 1). Some of the authors such as Kodenchery and Nair (1972), Hayashi and Kawashima (1982b), reported an immediate increase in respiration after the irradiation, which may be due to the mobilization of reducing sugars from starch, and to an acceleration of oxidation of respiratory substances, coupled to phosphorylation of ADP.

In the *variety Metal* tubers, the respiration rate was significantly higher than that of the *variety*, *Desire* and this increase in respiration may be due to fungal infection, which makes the respiratory rate rise in the infected tissues. It was observed that the respiration rate increased to a maximum within one month of the storage period, as shown in Fig. 2, then decreased in the second month in the control, and in the irradiated Metal variety tubers stored at 20–25 °C. The tubers irradiated with a dose of 50 Gy showed higher values of respiration. The control tubers gave lower values. At 5 °C the control and

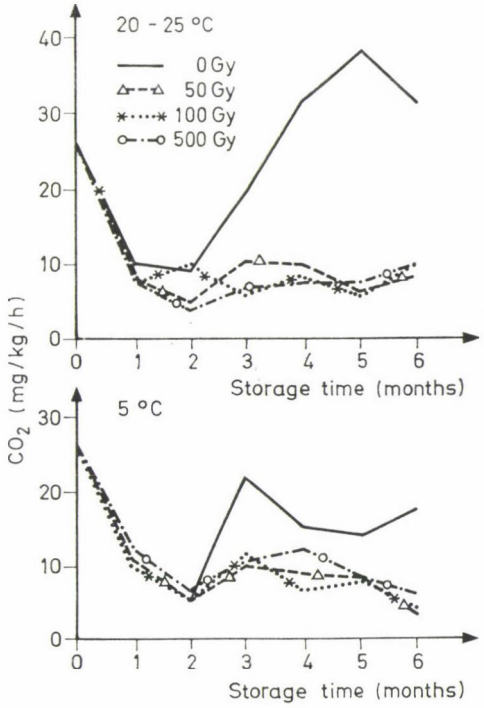

Fig. 1. Effect of irradiation and temperature on respiration of potato *variety Desire* in function of storage time

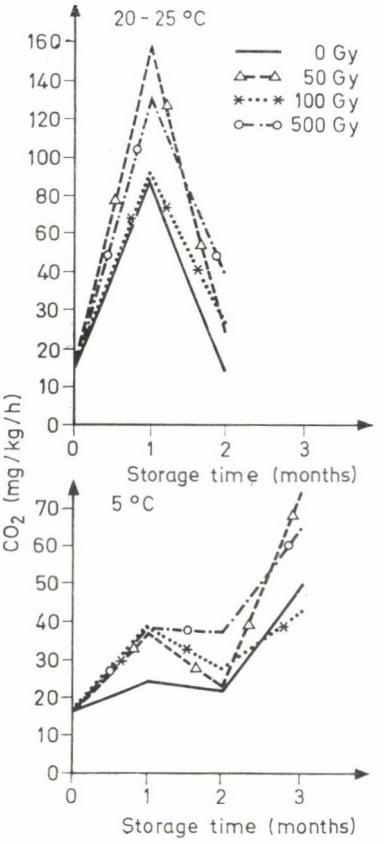

Fig. 2. Effeet of irradiation and temperature on respiration of potato *variety Metal* in function of storage time

irradiated *variety Metal* tubers showed an increase in respiration during 3 months of storage, but the lowest values were shown by the control, and higher values were shown by tubers irradiated with 50 and 100 Gy. It may be assumed that respiration played an important role in the accumulation of sucrose and the loss in weight of the sweet potato roots. The influence of oxygen on the increase of the sucrose content supports this assumption (Hayashi and Kawashima 1982b).

Total sugar content

It was found that at the end of the 2nd month following irradiation, the irradiated Desire variety tubers stored at 5 °C and *variety Metal* stored at 5 °C and 20–25 °C, had a higher content of sugars than did the unirradiated ones. This increase in sugars reached a maximum in the 2nd month of the storage period in the *variety Desire* tubers stored at 5 °C (35.3, 48.3, 46 and

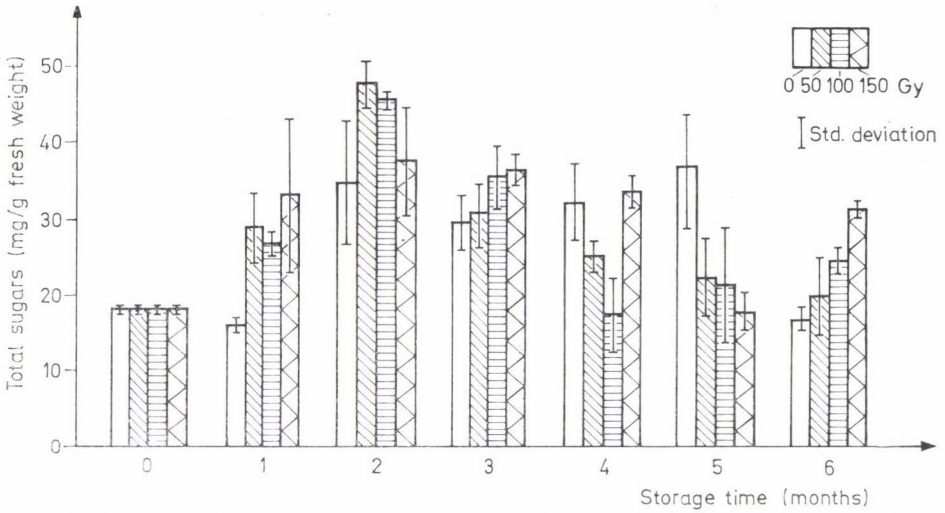

Fig. 3. Effect of irradiation and temperature on total sugar content of potato *variety Desire* in function of storage time. Temperature 5 °C

38 mg/g in 0, 50, 100 and 500 Gy treatments, respectively), and in the 3rd month for control, 50 Gy and 100 Gy treatment of Metal variety stored at 5 °C (32.6, 37.6 and 35 mg/g in 0, 50 and 100 Gy treatments, respectively), as shown in Figs. 3, 5. In the *variety Desire* tubers stored at 20–25 °C the sugar content was higher in the control tubers (49 mg/g) than in the irradiated ones (Fig. 4). A decrease in sugar content of the *variety Desire* was observed

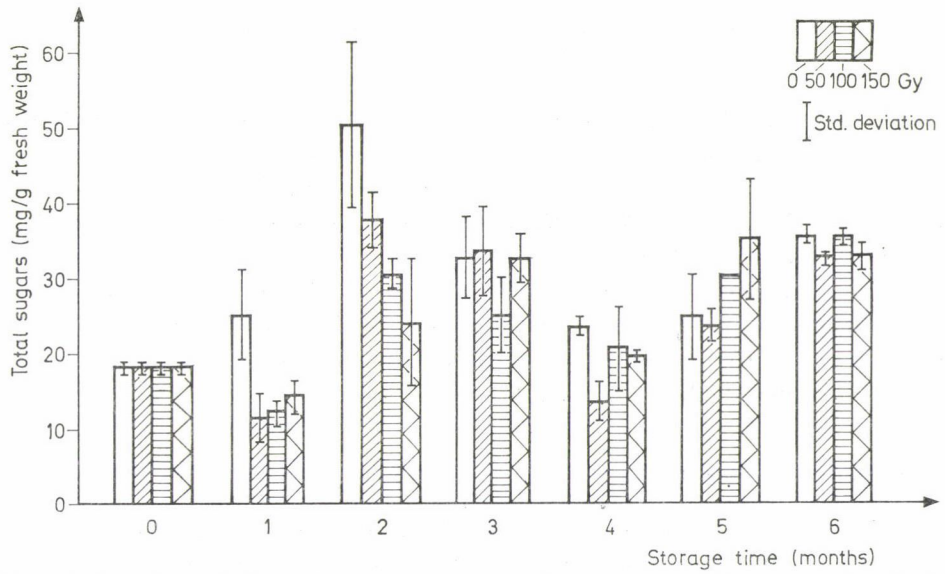

Fig. 4. Effect of irradiation and temperature on total sugar content of potato *variety Desire* in function of storage time. Temperature 20–25 °C

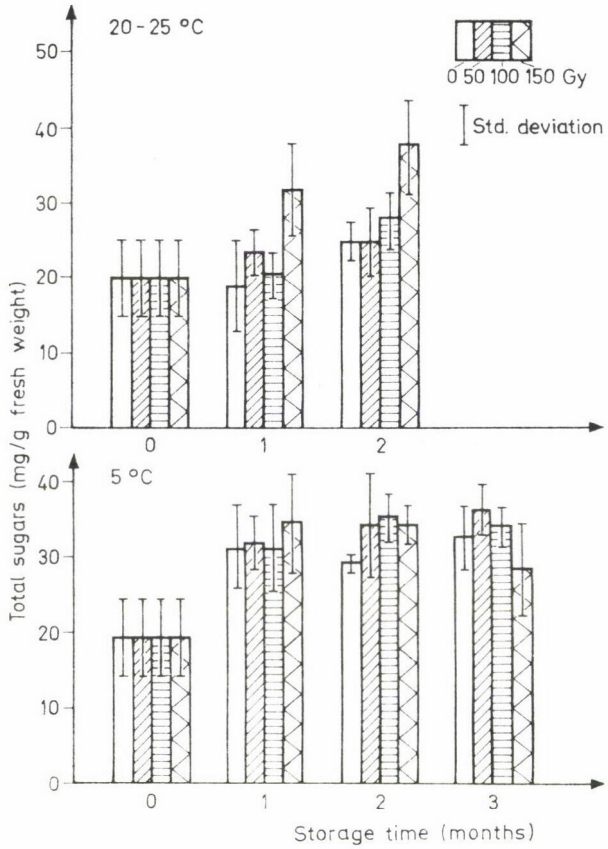

Fig. 5. Effect of irradiation and temperature on total sugar content of potato *variety Metal* in function of storage time

in the 3rd and 4th months of storage, followed by a slight increase. It was found that the sugar content was higher in the tubers stored at 5 °C than in the tubers stored at 20–25 °C, which finding is similar to the reports of Schwimmer et al. 1958, Jaarma 1968, Ohad et al. 1971, Dwelle and Stallknecht 1978, and Workman et al. 1979.

The irradiated tubers showed higher values of sugar accumulation than did the control, except for the control *variety Desire* tubers stored at 20–25 °C, which agrees with Clutier at al. 1961, Kodencherry and Nair 1972, Hayashi and Kawashima 1982a, 1983, and Beczner 1983.

Reducing sugar content

Reducing sugar content increased during the storage time and it reached a maximum (208% in 50 Gy, 188% in 100 Gy and 172% in 500 Gy treatments in percent of the control) in the 5th month of the storage period, but later de-

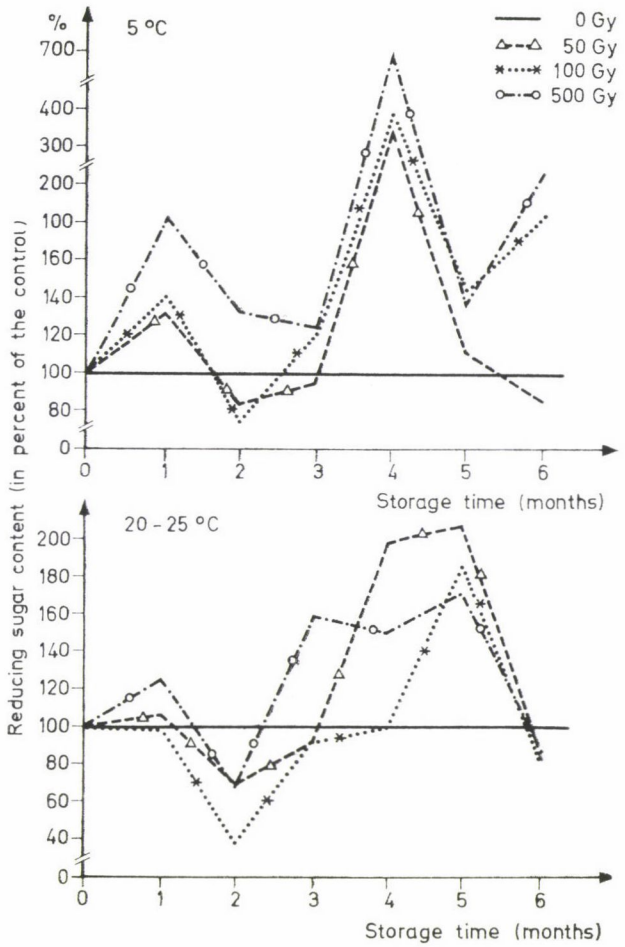

Fig. 6. Effect of irradiation and temperature on reducing sugars content of potato *variety Desire* in function of storage time

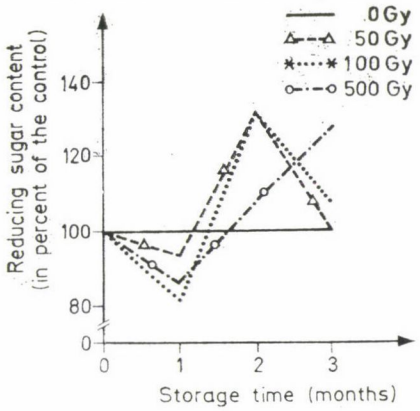

Fig. 7. Effect of irradiation and temperature on reducing sugars content of potato *variety Metal* in function of storage time. Temperature 5 °C

creased in the *variety Desire* tubers stored at 20–25 °C. At 5 °C the reducing sugar content showed a maximum value (350% in 50 Gy, 400% in 100 Gy and 700% in 500 Gy irradiated samples in percent of the control) in the 4th month of storage and then decreased. It was also observed that these values increased along with the increase of the irradiation dose (Fig. 6).

It was found, in *variety Metal* tubers stored at 5 °C, that the content of reducing sugars reached a maximum (131% in 50 Gy and 100 Gy treated samples in percent of the control) in the 2nd month of storage and then decreased (Fig. 7). The increase in reducing sugars was highest in the tubers stored at a lower temperature (5 °C), which closely agrees with Dwelle and Stallknecht (1978), who reported that total and reducing sugar contents were highest in tubers stored at 1.7 °C and lower in tubers stored at 10 °C, but later the content of both total and reducing sugars gradually decreased. It also concurs with the observation of Umeda (1979), who noted that after irradiation the reducing sugar content increased remarkably, and who attributed this to the transfer from high temperature to low temperature. Between the two different methods used in the total and reducing sugars, we observed that the sugars increased more in the irradiated tubers than in the control ones, and also more in the tubers stored at 5 °C than in those stored at 20–25 °C.

Starch content

It was observed that the starch content in both varieties of potato with different treatments decreased during the storage time. It fell slightly in the Desire tubers stored at 20–25 °C, to levels of 16%, 11.5%, 11.4% and 13.2% in the last month of storage in 0, 50, 100 and 500 Gy treatments, respectively, as shown in Fig. 9. However, a little amount of starch converted to sugar, which was determined by the lower levels of sugars observed in the *variety Desire* stored at 20–25 °C, than in those stored at 5 °C. In the Desire tubers stored at 5 °C, the starch content decreased much more than in those stored at 20–25 °C. It was 15.7%, 14%, 11.5% and 10% in the last month of storage in 0, 50, 100 and 500 Gy treatments, respectively (Fig. 8). This decrease in starch content concurred with the increase in sugar content. It was noted that the decrease in starch content was greater in the irradiated tubers than in the control ones, except for those that had the 50 Gy treatment. It was also noted that the increase in sugars was higher in irradiated tubers than in the control ones, which proves the conversion of starch into sugars, which agrees with Ohad et al. (1971), Isherwood (1976) and Workman et al. (1979). In the case of *variety Metal* tubers, these also showed a higher decrease of starch content in tubers stored at 5 °C than in those stored at 20–25 °C during the 3 months of storage. The control tubers stored at 20–25 °C showed a higher mean starch value (12%) than did the irradiated ones (8.4% in 50 Gy, 11% in 100 Gy and 9% in 500 Gy treatment) (Fig. 10).

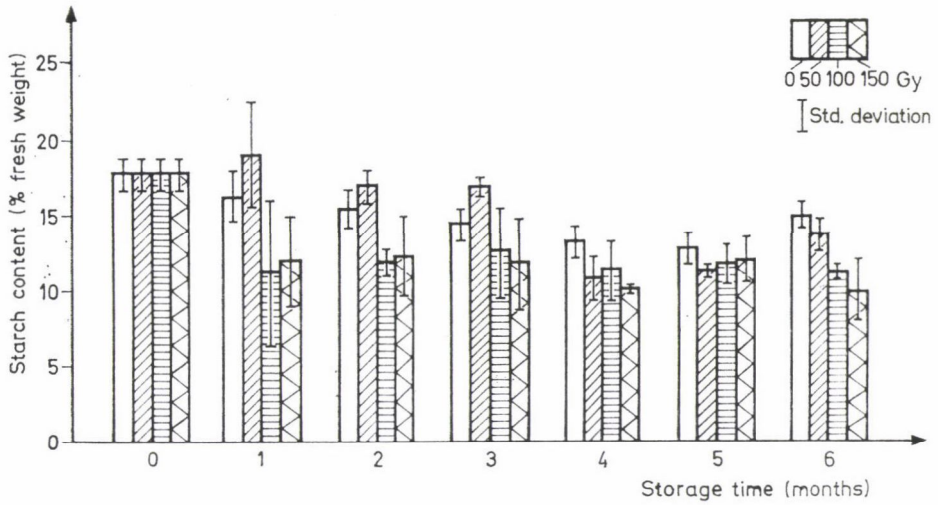

Fig. 8. **Effect of irradiation and temperature on starch content of potato *variety Desire* in function of storage time. Temperature 5 °C**

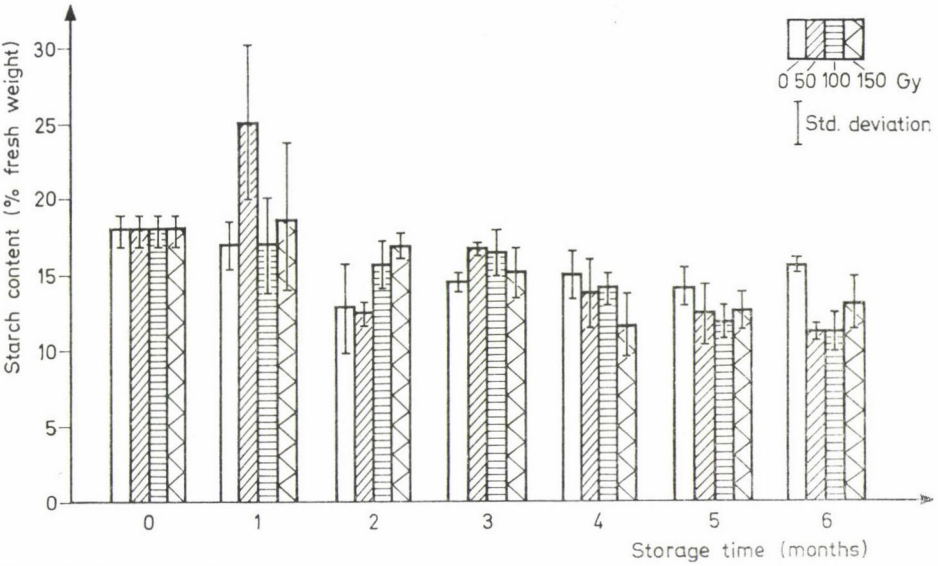

Fig. 9. **Effect of irradiation and temperature on starch content of potato *variety Desire* in function of storage time. Temperature 20–25 °C**

Conclusion

It can be concluded that gamma irradiation effects the respiratory rate of potatoes, depending on the variety and storage conditions. In this experiment the first measurements of respiration were carried out within one month after irradiation. It was observed that the control *variety Desire* samples

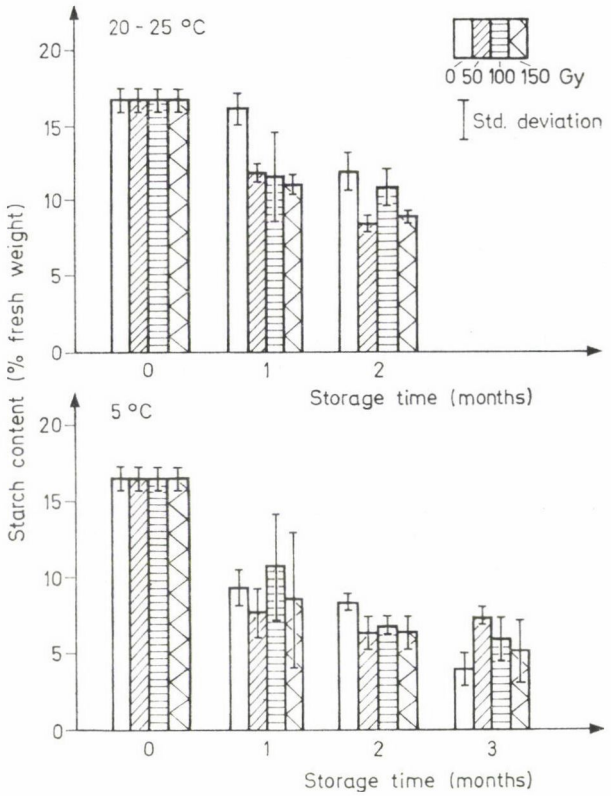

Fig. 10. Effect of irradiation and temperature on starch content of potato *variety Metal* in function of storage time

showed a higher respiration in comparison with the irradiated tubers. The respiration rate decreased, and then increased. Higher values of respiration were observed in the control samples stored at 20–25 °C (37.9 mg CO_2/kg/h) than in those stored at 5 °C (21.5 mg CO_2/kg/h), and in all treatments the respiration rate decreased during the storage time, which may be due to the effect of irradiation on the enzymes. In the *variety Metal* tubers the respiration rate was significantly higher than in the *variety Desire*. It increased to a maximum within one month of storage, and then decreased. The lower values were given by the control tubers. This increase in respiration may be because of fungal infection. The *variety Metal* tubers stored at both storage temperatures (5 °C and 20–25 °C) were infected by *Fusarium ventricosum*, which was difficult to compare between the two varieties of potato.

The experimental results indicated that the sugar content accumulated in the irradiated tubers of *variety Metal* and of *variety Desire* stored at 5 °C. It was higher in the irradiated samples than in the control ones, except for the Desire tubers stored at 20–25 °C, and accumulated more in tubers stored at

low temperature, depending on the variety and irradiation dose. Reducing sugars increased during storage time and it reached a maximum in the 4th and 5th month of the storage period, then decreased. The increase in reducing sugars was also highest in tubers stored at lower temperature (5 °C). It can be concluded that the starch content decreased much more in irradiated potato tubers than in unirradiated ones, also that it decreased more in tubers stored at lower temperature. The decrease in starch content concurred with the increase in sugar content, which proves the conversion of starch into sugars. With the increase of sugar content and decrease in starch content, a rise in respiratory activity is expected, so a high amount of sugar will be used and material will be lost from the composition of tubers due to respiration. It was observed that, in the control samples, the increase in respiration accompanied a decrease in total sugars. Therefore, it is necessary to control the atmosphere surrounding the stored product to reduce the respiratory rate or the inhibiting ripening process.

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 69–77 (1991)

CHARACTERIZATION OF BEAN AND MAIZE GENOTYPES BY PHOTOSYNTHETIC PARAMETERS DURING THE START OF ILLUMINATION

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(Received: 7th August 1989; accepted: 29th September 1989)

During the first 60 minutes of the illumination the start of CO_2 assimilation, the levels of malate, sucrose, starch were studied in isolated leaves deepoxidating violaxanthin quickly (XQ genotypes) and slowly (XS genotypes) of bean and maize plants growed in phytotron for 40 days. The maximal acceleration of the CO_2 assimilation is less and the lag phase of the CO_2 assimilation is longer in the XQ than in the XS genotypes because of the slower start of the non-cyclic electrontransport in the XQ genotypes.

In the XQ genotype of the C_3 type bean reaches the initial sucrose peak sooner than the XS genotype because of the quicker activation of sucrose synthesis. The initial change of the malate level may not be in tight correlation with the induction of CO_2 assimilation.

In the XQ genotype of the C_4 type maize the lower acceleration of CO_2 assimilation and the less initial decline of malate level show that the non-autocatalytical induction produced from internal carbon source is not so effective than in the XS genotype. The initial decline of sucrose may indicate that sucrose is one of the internal carbon sources besides aspartate and malate in the C_4 maize plant.

Keywords: bean, maize, photosynthesis, genotypes

Introduction

The different performances of the genotypes within a genus or a species are well-known and especially available in the crop plants. The differences of genotypes may be in connection with the modulation of metabolic pathways. We can observe such as variations in the photosynthetic pathways of genotypes (the C_3 and C_4 species of *Panicum* genus [Downton 1975]), and the variation of dry matter production (genotype pairs of bean, maize, sunflower being different in quick [XQ] and slow [XS] deepoxidating of violaxanthin during the induction period of photosynthesis [Maróti 1986]).

Our aim was to study the genotypical differences between the XQ and the XS genotypes of the C_3 type bean and the C_4 type maize through the rate of CO_2 assimilation, distribution of malate, sucrose and starch. The plants were grown at medium low photosynthetic photon flux density and short light-dark period. The lag phasis of the induction period of photosynthesis is

caused mainly by the changes of concentration of intermediers (Leegood and Walker 1980). It is known that light and dark activate a number of chloroplastic enzymes (Anderson 1979; Scheibe and Jacquot 1983; Karabourniotis, Manetas and Gavalas, 1983). The phosphofructokinase-2 (EC 2. 7. 1. 105) (Paz, Xu and Black, 1985), playing on outstanding role in carbohydrate metabolism in the photosynthetic induction, may be activated by light (Preiss 1987).

The light-activated changes of the sucrose phosphate synthesis (SPS, EC 2.3.1.14) are important in the regulation of photosynthetic synthesis of sucrose (Sicher and Kremer 1985). During the induction period the intermediers of photosynthetic carbon reduction cycle (PCRC) in C_3 plants are built up autocatalytically (Leegood and Walker 1980; Usuda 1986). The start of photosynthesis is not accompanied by general intermedier synthesis, but certain intermediers are perturbated, which is caused by light activations of the enzymes.

Above all the fructose-1.6-bisphosphate phosphatase (EC 3.1.3.11) and the riboluse-1.5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39) limit the carbon flow in the PCRC (Stitt, Wirtz and Heldt 1980).

The carbon metabolism is under fructose-2.6-bisphosphate regulation during the induction period (Smyth and Black 1984; Paz and Xu 1985; Preiss 1987). The reducing power (NADPH) and energy (ATP), necessary to the synthetic reactions in cytoplasma, are produced in light in the chloroplasts. The envelope membrane of the chloroplast is impermeable for these molecules therefore the dicarboxylate translocator, carrying oxalacetate to the site of malate, is very important. The malate is the main indirect carrier of the carbon among chloroplast, cytoplasm, mitochondrium and peroxisoma (Heber 1974; Tolbert 1979; Scheibe, Wagenpfeil and Fischer 1986; Graham and Chapman 1979).

The ribulose-1.5-bisphosphate and other intermediers have a further non-autocatalytic synthesis that shortens time necessary to reach the steady-state operation of PCRC (Usuda 1986). The level of C_3 compounds (pyruvate, alanin, PEP) rise while the level of C_4 compounds (malate and aspartate) decline in C_4 cycle during the first 30 seconds of illumination (Usuda 1986).

The increase of the quantity of carbon surpasses carbon deriving from the CO_2 fixation. It refer to an internal carbon source (Furbank and Leegood 1984; Usuda 1985). Aspartate, being a very close metabolic connection with malate, is CO_2 pool and reduces the duration of photosynthetic induction (Creach, Michel and Thibault 1974).

The malate plays role not only in the intracellular transport but in carrying energy and carbon skeleton between mesophyll and bundle sheath cells (Slack, Hatch and Goodchild 1969; Coombs 1976).

The rate of the deepoxidation of violaxanthin (i.e. the rate of developing of protongradient in thylakoids) quicker, the rate of quenching of chlorophyll-a fluorescence in the M-T period (i.e. start of the non-cyclic electrontransport)

and the rate of O_2 evolution is slower in the XQ genotypes than in the XS genotypes (Pataky and Maróti 1985; Walker 1985; Maróti 1986) during the induction period of photosynthesis. The XQ genotypes have lower dry matter, leaf thickness and growth rate, their water content are higher than those of XS genotypes (Maróti and Margóczi 1984; Margóczi and Maróti, 1985). The quantity of oppressed membrane in the chloroplasts of the XQ genotypes is greater, the number of grana, the sizes of the loculi are less than in the XS genotypes (Pataky and Maróti 1985; Maróti 1986).

Materials and methods

The comparison of photosynthetic induction were carried out on the XQ and the XS genotypes of bean (*Phaseolus vulgaris* L. 'Cherokee') as well as on the XQ ('F_2' line) and the XS ('P165' line) genotypes of maize (*Zea mays* L. 'Pioneer'). The plants were growed for 40 days in 600 cm^3 plastic pots in the mixture of sand-perlit (1 : 1) in phytotron where the CO_2 content in the air 330 $\mu mol \cdot mol^{-1}$, the saturation deficit of water vapour in the air 8.4 $mmol \cdot mol^{-1}$, the temperature 23 °C were. The 3 light treatments were: medium light (ML): 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD) and 16 h–8 h light dark period (LDP): low light (LL): 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD and 16 h–8 h LDP; short period light (SPL): 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD and 30 min–15 min LDP. The light sources were fluorescent tubes (Tungsram F33 types).

The ML and the LL plants were kept in darkness for 8 hors, the SPL plants were kept in darkness only for 30 minutes then their totally developed leaves (bean: 1st trifoliate leaf, maize: 5th leaf) were cut. The isolated leaves were illuminated with 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD light in humid, 26 °C, 340 $\mu mol \cdot mol^{-1}$ CO_2 concentration of air for 0, 2, 5, 15, 30, 60 minutes, then they were fixed in liquid air and lyophilized.

The malate (Hohorst 1970), the sucrose and the starch content (Handel 1968; Dubois, Gilles, Hamilton, Rebers and Smith 1956) were determined. We measured the temporal changes of the rate of CO_2 assimilation with infrared gas analyser (VEB Junkalor: Infralyt 4.). The calculations were carried out after Long and Hallgren (1985); Janac, Catsky and Jarvis (1971); Caemmerer and Farquhar (1981).

The acceleration of CO_2 assimilation (a) means the increment of the function of CO_2 assimilation rate (A) plotted against time (t):

$$a = \frac{dA}{dt}$$

Results

The maximal acceleration and the lag time of the start of CO_2 assimilation (Fig. 1).

Bean

The maximal acceleration of CO_2 assimilation is significantly lower in the XS genotype growed at the LL and in the XQ genotype growed at the SPL. At each of the light treatments the maximal acceleration is lower with the exception of the LL and the lag phase is higher in the XQ than in the XS genotype.

Maize

The LL and the SPL treatments stronger increase the maximal acceleration of CO_2 assimilation in the XS than in the XQ genotypes of maize. At almost each of the treatments the maximal acceleration is lower in the XQ genotype and the lag phase is higher than in the XS genotype. Levels of malate, sucrose and starch during the first 60 minutes of the illumination (Figs. 2., 3).

Bean

We could observe no significant changes in the malate content of the leaf in the interval of 0–15 minutes.

After the initial peak a constant rise could be observed in the sucrose level of the leaf. The XQ genotype reaches the initial sucrose peak in shorter duration (2–5 minutes) than the XS genotype (5–15 minutes).

There is not significant change in starch level of the leaf at the first 60 minutes of the illumination.

Maize

After an initial decline the malate level of the leaf rises. The duration of the initial decline is shorter in the leaves of the LL and the SPL plants (5 minutes) comparing to the ML plants (15 minutes). The decline is less in the LL and similar in the SPL plants than the ML plants. The rate of the rise of the malate level, preceded an initial decrease, is slower in the XQ than the XS plants.

After an initial decline (5 minutes) the sucrose content of the leaf constantly rises slower in the XQ then in the XS genotype. There is not any significant change in starch content of the leaves during the first 60 minutes of the illumination.

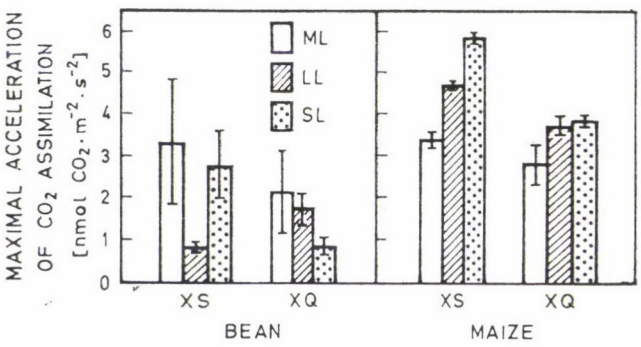

Fig. 1. The maximal acceleration of the start of CO_2 assimilation in the totally developed leaves of bean and maize XS as well as XQ genotypes grown at ML, LL and SPL light regimes during the first 60 minutes of the illumination with light of 800 μmol $\cdot$ $m^{-2} \cdot s^{-1}$ PPFD

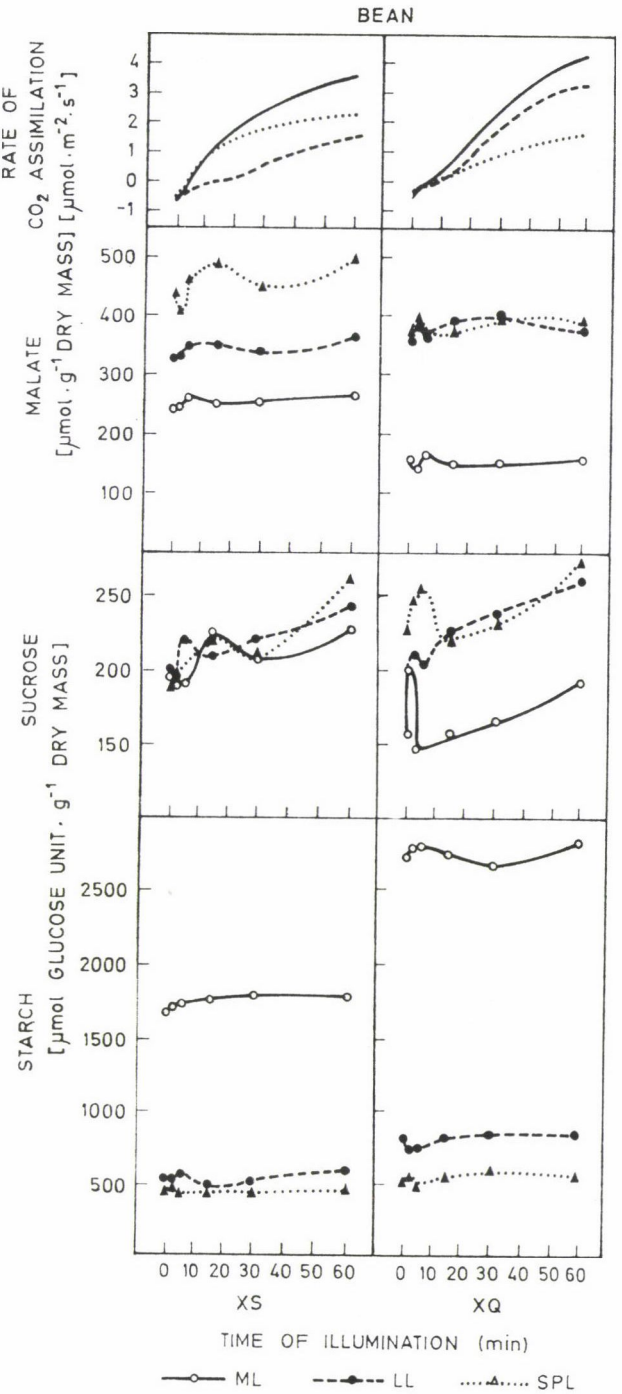

Fig. 2. The CO_2 assimilation rate and the levels of malate, sucrose and starch of the totally developed leaves of bean XS as well as XQ genotypes grown at ML, LL and SPL light regimes during the first 60 minutes of the illumination with light of 800 μmol · m^{-2} · s^{-1} PPFD

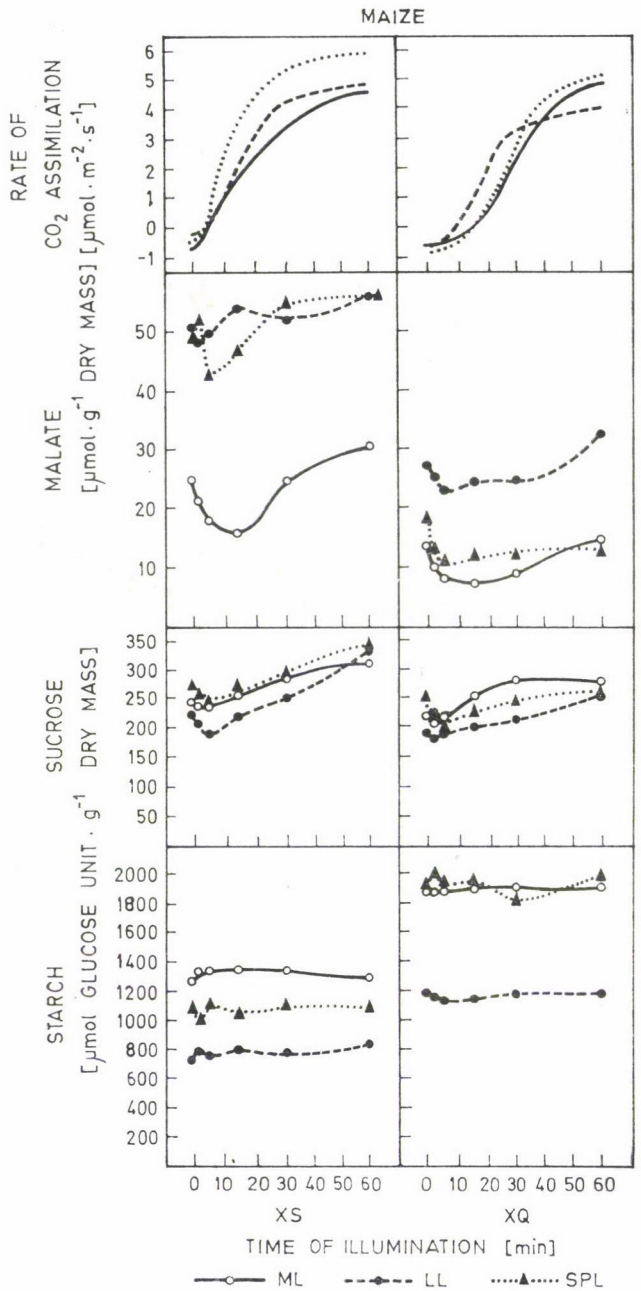

Fig. 3. The CO_2 assimilation rate and the levels of malate, sucrose and starch of the totally developed leaves of maize XS as well as XQ genotypes grown at ML, LL and SPL light regimes during the first 60 minutes of the illumination with light of 800 μmol · m^{-2} · s^{-1} PPFD

Discussion

The CO_2 assimilation starts with a lag phase in the C_3 bean and the C_4 maize as well. The bean's lag phase increases, their maximal acceleration of CO_2 assimilation decreases while the maize's lag phase decreases, and their maximal acceleration of CO_2 assimilation increases at the LL and the SPL treatments comparing to the ML treatment. These phenomena show that the induction of C_3 and C_4 photosynthesis are quite different (Leegood and Walker 1980; Usuda 1986).

In the C_3 bean the rate of the autocatalytical building up of intermediers of PCRC may reduce in the LL and SPL plants. The main cause of this reduction may be the lower activity of the light-induced enzymes in the plants growed at the LL and SPL.

In the C_4 maize a non-autocatalytical building up of intermediers (Usuda 1986) accelerates the induction. The building up of intermediers derives not from the CO_2 fixation but from an internal carbon source that is vacuolar aspartate (Creach et al. 1974), and malate or might be sucrose. It seems the role of malate and sucrose is supported by the quicker decrease of the level of malate and sucrose in the LL and the SPL maize plants that may be in close connection with the shorter lag phase and the higher maximal acceleration of CO_2 assimilation in the leaves of genotypes growed at LL and SPL light regimes.

In the C_3 bean the initial rise of malate level slows down the induction with sucking the intermediers of PCRC.

In the C_4 plants the decarboxylation, catalysed by malic enzyme (EC 1.1.1.40), accounts the initial decline of malate level. It is also supported by the rise of levels of C_3 compounds (PEP, pyruvate, alanin) in the leaves during the first minutes of induction (Usuda 1985).

In the XQ genotypes the quicker deepoxidation of violaxanthin, the slower quenching of fluorescence in the M-T period (Maróti, 1986) the lower rate of 02 evolution are connected with the lower maximal acceleration but the greater lag phase of the CO_2 assimilation comparing to the XS genotypes. In the XQ genotypes the protongradient develops quicker, which is indicated by the quick deepoxidation of violaxanthin, the non-cyclic electrontransport and the CO_2 assimilation starts slower than in the XS genotypes.

In the starch level of bean and maize leaves we could not find any significant changes because these changes in the first 60 minutes are likely negligible to the initial starch content.

Bean (Fig. 2)

The initial sucrose peak of XQ genotypes shows the start of the sucrose synthesis at the beginning of the illumination. The key enzyme is the light-activated SPS (Sicher and Kremer 1985). The cause of the decline followed the

sucrose peak may be the quickly synthetized fructose-2,6-bisphosphate, that ceases the carbon flow toward sucrose and stimulates the starch synthesis in the chloroplast (Preiss 1987). The sucrose synthesis will start again and will rise if DHAP is formed in excess and it begins to export from the chloroplast to the cytoplasm. The sucrose peak appears sooner and sharper in the leaves of the XQ than those of the XS bean genotypes. Therefore the SPS may be activated sooner in the leaves of the XQ than of the XS. Nevertheless the fructose-2,6-bisphosphate modulates the photosynthetic enzymes of the two genotypes on different way. The metabolit pools rearrange after a temporal inhibition of the production of metabolits of tricarboxylic acid cycle and may rise the malate level at the beginning of the induction (Graham and Chapman 1979) in the leaves of the C_3 bean.

Maize (Fig. 3)

The lower maximal acceleration of the CO_2 assimilation may be in close correlation with the lower rate of O_2 evolution (Pataky and Maróti 1985). The longer lag phase and the lower maximal acceleration of CO_2 assimilation and the slower quenching of fluorescence in the M-T period in the XQ genotypes show the slower start of the non-cyclic electrontransport comparing the XS genotypes of maize. The slower start of CO_2 assimilation, the less decline of the malate level show that the non-autocatalytical induction depending on an internal carbon source functioning not so effectively in the XQ genotypes as in the XS genotypes of the C_4 maize.

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Acta Agronomica Hungarica, 40 (1–2), pp. 79–85 (1991)

Plant Cultivation

OPTIMUM NPK RATIO OF A PLANTED GRASSLAND

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(Received: 4th November, 1988; accepted: 9th January, 1989)

On a planted grassland with high production potential supplied with 300 and 400 kg/ha N, respectively, the optimum NPK ratio = 1 : 0.08 : 0.17 and 1 : 0.06 : 0.13 — set in with P 25- and K 50 kg/ha active agent applied. In the experiment at the two N levels the gradually and parallel increasing PK doses increased the yield by 139—166 and 161—191 percent, respectively, decreased the percentage N content and increased the P and K contents of the grassland, changed the ratios of the nutritive element pairs, and were utilized with a decreasing tendency.

Keywords: grassland, fertilization, optimum NPK ratio.

Introduction

NPK fertilization will be most efficient and economical when adjusted to the given in this case grassland and soil (Bánszki 1971). The optimum level of N fertilization is determined by the production potential of the grassland, the composition of the plant stand, the way the grassland is used, along with the water regime of the soil and the amount of local precipitation. Besides the yield and nutrient demand of the grassland the necessary quantities of P and K fertilizer depend on the PK status of the soil as well as on its availability and utilization.

For grasslands different NPK ratios are considered optimum: e.g. 1 : 0.3 : 0.1 by Mihajlicsenko (1968); Bánszki (1971, 1973) suggested varying ratios at different N levels and for different soils and grassland types, e.g. on alkali soil for irrigated meadow foxtail 1 : 0.25 : 0.25 at N 320 level, for irrigated meadow fescue — narrow leaved blue grass 1 : 0.17 : 0.17 at N 480; Barcsák et al. (1978) found the ratio of 1 : 0.4 : 0.4 to be optimum; Bedford (1979) for hay production suggested 1 : 0.20 : 0.63 NPK ratio at N 448 kg/ha; according to Vinczeffy (1983) 1 : 0.23 : 0.43 is the best ratio.

In the experiment the most efficient P and K fertilizer doses were to be found for two high rates of N at a P/K ratio of 1 : 2, in order to determine the optimum NPK quantities and -ratios of the grassland on a given soil and area.

Table 1

Treatments in the experiment

Number of treatments	Fertilizer active agent, kg/ha			Fertilizer active agent ratios
	N	P_2O_5	K_2O	$N : P_2O_5 : K_2O$
1.	—	—	—	— — —
2.	300	25	50	1 : 0.08 : 0.17
3.	300	50	100	1 : 0.17 : 0.33
4.	300	75	150	1 : 0.25 : 0.50
5.	300	100	200	1 : 0.33 : 0.67
6.	300	125	250	1 : 0.42 : 0.83
7.	300	150	300	1 : 0.50 : 1.00
8.	400	25	50	1 : 0.06 : 0.13
9.	400	50	100	1 : 0.13 : 0.25
10.	400	75	150	1 : 0.19 : 0.38
11.	400	100	200	1 : 0.25 : 0.50
12.	400	125	250	1 : 0.31 - 0.63
13.	400	150	300	1 : 0.38 : 0.75

Materials and methods

Between 1975 and 1980 at the grassland experiment station of the Debrecen University of Agricultural Sciences, Hajdúszoboszló, we studied the effects of various amounts and ratios of P and K at 300 and 400 kg/ha N levels in order to determine the optimum of NPK. The treatments of the experiment are shown in Table 1.

The experiment was laid out in random block design with 4 replications, on plots of 24 m^2 each cut three times a year. The area was flat, about 100 m above sea level.

The composition of the grass mixture was meadow fescue (*Festuca pratensis* Huds.) Szarvasi-54, 14-, dactylis (*Dactylis glomerata* L.) Szarvasi-51, 8-, rye-grass (*Lolium perenne* L.) "G-658" 6-, Hungarian brome grass (*Bromus inermis* Leyss) Szarvasi-52 6-, blue grass (*Poa pratensis* var. *latifolia* L.) "G" széleslevelű 4-, red fescue (*Festuca rubra* L.) "G" 3-, timothy (*Phleum pratense* L.) "G" 3-, clover (*Trifolium repens* var. *giganteum* Lagr.) 2 kg/ha.

The soil was a lowland chernozem with lime deposits; the results of a soil analysis of the 0—20 cm layer before the experiment was set up were: pH (KCl) 6.2; K_A 44; total salt % 0.02; humus 3.4; nutrient status in ppm: $NO_3 + NO_2$ 1.7; AL-soluble P_2O_5 44 and K_2O 239; Mg 575; Na 50; Zn 1.4; Cu 5.3; Mn 100; SO_4 11.7. According to the MÉM NAK categories the soil was well-supplied with humus, but poorly with phosphorus and moderately with potassium.

During the time of the experiment, precipitation and temperature were somewhat below the 50-year average (583 mm and 10.0 °C); 1976 and 1979 were particularly dry years.

In the experiment 34% ammonium nitrate, 18% granular superphosphate and 40% KCl were used. The P and K fertilizers were distributed in autumn all at one time and the N fertilizer was supplied in 3 equal doses before cutting.

We measured the fresh crop and the dry matter output, and from the plant samples determined the macroelements. The experiment results were evaluated by variance- and regression analyses.

The experiment was analysed and appreciated on the basis of the six-year results of the grassland.

Results and discussion

Dry matter output of the grassland and efficiency of fertilization

The PK treatment increased the dry matter output to 239—266% at the N 300- and to 261—291% at the N 400 level, compared to the control

(Table 2). With identical PK supplies, the difference in dry matter output between the N levels is significant almost in each treatment, while in N quantities only with greater quantitative differences of PK treatments (Fig. 1).

The yield surplus per 1 kg active agent is the most favourable with the PK 25—50 kg/ha dose at both N levels, after which the increasing rates of PK cause a decreasing tendency in yield.

Owing to the lack of precipitation the N 400 kg/ha dose was not as efficient as the N 300. The most efficient treatment of the experiment was NPK 300—25—50 kg/ha; the optimum NPK ratio is thus 1 : 0.08 : 0.17. In this treatment 56.9 kg NPK active agent was required to produce 1 ton dry matter surplus.

Table 2

Dry matter outputs and efficiency of the treatments on the average of 1975—80
(Hajdúszoboszló)

Number of treatments	Dry matter output			Surplus yield per 1 kg mixed active agent, kg	Active agent required to produce 1 t surplus dry matter (kg)			
	t/ha	%	D		Total	N	P_2O_5	K_2O
1.	4.75	100	—	—	—	—	—	—
2.	11.34	239	6.59	17.6	56.90	45.52	3.78	7.60
3.	11.53	243	6.78	15.1	66.37	44.25	7.37	14.75
4.	12.31	259	7.56	14.4	69.44	39.68	9.92	19.84
5.	12.27	258	7.52	12.5	79.79	39.90	13.30	26.59
6.	12.45	262	7.70	11.4	87.66	38.98	16.22	32.46
7.	12.63	266	7.88	10.5	95.18	38.07	19.04	38.07
$y_1 =$	$10.76 + 0.57x - 0.04x^2$							
$R_1 =$	0.96							
8.	12.40	261	7.65	16.1	62.09	52.18	3.13	6.78
9.	12.05	254	7.30	13.3	75.34	54.79	6.85	13.70
10.	12.89	271	8.14	13.0	76.78	48.91	9.29	18.58
11	13 03	274	8.28	11.8	84.54	48.31	12.08	24.15
12.	13.43	283	8.68	11.2	89.29	46.09	14.40	28.80
13.	13.82	291	9.07	10.7	93.72	44.10	16.54	33.08
$y_2 =$	$12.13 + 0.07x + 0.04x^2$							
$R_2 =$	0.95							
LSD 5%	0.55	12						

In spite of its poor P- and medium K status, the soil proved to be a good PK supplier, since the optimum set in with the lowest rate of PK.

Specific nutrient content

The specific nutrient content varied with the treatments (Table 3). It was influenced by a decrease in the number of leguminous plants, in the grass mixture in response to N fertilization, and by the change of the grass-

Table 3

Specific values of nutrients in terms of dry matter percentage on the average of 1975—80 (Hajdúszoboszló)

Number of treatments	Values of nutrients in terms of dry matter %					
	N %	%	P %	%	K %	%
1.	2.01	100	0.28	100	2.12	100
2.	2.57	128	0.23	82	2.10	99
3.	2.34	116	0.24	86	2.18	103
4.	2.30	114	0.25	89	2.23	105
5.	2.27	113	0.26	93	2.27	107
6.	2.26	112	0.27	96	2.32	109
7.	2.25	112	0.28	100	2.27	112
$y_1 =$	$2.72-0.21x+0.02x^2$		$0.22+10x+0x^2$		$2.04+0.07x-0.003x^2$	
$R_1 =$	0.96		1.00		0.99	
8.	0.96	143	0.22	79	2.05	97
9.	2.43	121	0.23	82	2.08	98
10.	2.45	122	0.24	86	2.20	104
11.	2.37	118	0.25	89	2.22	105
12.	2.34	116	0.26	93	2.25	106
13.	2.32	115	0.27	96	2.28	108
$y_2 =$	$3.09-0.33x+0.03x^2$		$0.21+0.01x+0x^2$		$1.95+0.09x-0.006x^2$	
$R_2 =$	0.92		1.00		0.98	
LSD 5%	0.19	9	0.02	7	0.22	10

legume ratio. The control gave good component values — due to Trifolium repens var. giganteum — particularly in respect of protein- and phosphorus content (Fig. 1).

The N content was significantly reduced by the increasing P- and K doses. There was a difference in N percentage between the two N levels. The P concentration was decreased by the N- and increased by the P fertilizer. In response to increasing rates of K fertilizer the percentage value of K increased, though mostly non-significantly.

Owing to the different tendencies of the specific nutrient contents the indices of the ratios of element pairs changed in the treatments compared to the control (Table 4).

Nutrient output of the grassland and utilization of fertilizers

The treatments resulted in a considerable surplus nutrient output (Table 5). Since in comparison to the control the specific content of nutritive elements — except phosphorus — gradually increased, the N- and K_2O output per ha rose at a higher rate than the yield.

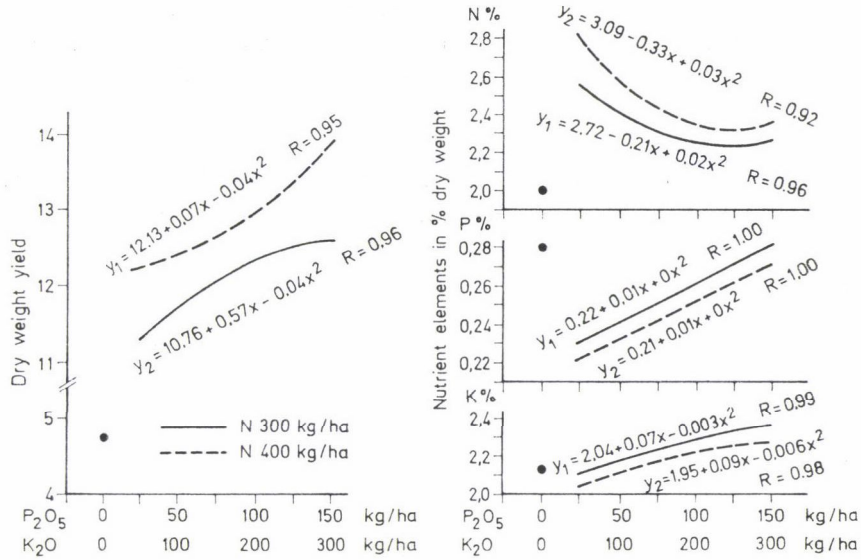

Fig. 1. Function curves for dry matter output and nutrient content (1975—1980, Hajdúszoboszló)

Table 4

Indexes of nutrient ratios on the basis of specific values on the average of 1978—80 (Hajdúszoboszló)

Number of treatments	Indexes of nutrient ratios									Equivalent K
	N/P	N/K	N/Ca	N/Mg	P/K	P/Ca	P/Mg	K/Mg	Ca/Mg	Ca + Mg ratio
1.	7.18	0.95	4.57	10.05	0.13	0.64	1.40	10.60	2.20	1.41
2.	11.17	1.22	7.34	11.68	0.11	0.66	1.05	9.55	1.60	1.50
3.	9.75	1.07	6.69	10.63	0.11	0.69	1.10	9.91	1.60	1.56
4.	9.20	1.03	6.39	10.95	0.11	0.69	1.20	10.62	1.71	1.61
5.	8.73	1.00	6.31	10.81	0.11	0.72	1.24	10.81	1.71	1.64
6.	8.37	0.97	6.11	11.30	0.12	0.73	1.35	11.60	1.85	1.69
7.	8.04	0.95	6.08	11.25	0.12	0.76	1.40	11.85	1.85	1.73
8.	13.05	1.40	8.70	13.05	0.11	0.67	1.00	9.32	1.50	1.51
9.	10.57	1.18	6.88	11.05	0.11	0.68	1.05	9.45	1.55	1.51
10.	10.21	1.11	7.00	11.14	0.11	0.69	1.10	10.00	1.60	1.58
11.	9.48	1.07	6.77	10.77	0.11	0.71	1.14	10.10	1.60	1.59
12.	9.00	1.04	6.50	11.14	0.12	0.72	1.24	10.71	1.71	1.63
13.	8.59	1.02	6.44	11.05	0.12	0.75	1.29	10.86	1.71	1.65

The N output increased by 283—305 and 307—373% and the K_2O content by 237—297 and 249—314%, respectively with the two N levels; the increase in P_2O_5 output at the two N levels was between 108 and 277%, compared to the control.

The utilization percentage of the fertilizers showed different values and tendencies. Utilization of the N fertilizer was 58—65% with 300 kg/ha, and 49—65% in the case of 400 kg/ha. Increasing PK doses slightly checked the utilization.

Increasing doses of P fertilizer were utilized with a decreasing tendency, from 126 to 34%. With 25 kg/ha P active agent more than 100% utilization was obtained at both N levels, probably due to the mobilization of the P content of the soil, or to some error in the experiment.

Table 5

N-, P- and K outputs and utilization percentages on the average of 1975—80 (Hajdúszoboszló)

Number of treatments	Nutrient outputs and utilization percentages of fertilizers (H%)											
	N				P_2O				K_2O			
	kg/ha	%	D	H%	kg/ha	%	D	H%	kg/ha	%	D	H%
1.	95.5	100	—	—	30.4	100	—	—	121.1	100	—	—
2.	291.4	305	195.9	65	60.1	198	29.7	119	286.9	237	165.8	332
3.	269.8	283	174.3	58	62.3	205	31.9	64	303.2	250	182.1	182
4.	283.1	296	187.6	63	70.2	231	39.8	53	331.1	273	210.0	140
5.	278.5	292	183.0	61	72.4	238	42.0	42	334.9	277	213.8	107
6.	281.4	295	185.9	52	77.2	254	46.8	37	348.6	288	227.5	91
7.	284.2	298	188.7	63	80.8	266	50.4	34	359.9	297	238.8	80
8.	355.9	373	260.4	65	62.0	204	31.6	126	306.3	253	185.2	370
9.	292.8	307	197.3	49	63.9	210	33.5	67	301.3	249	180.2	180
10.	315.8	331	220.3	55	70.9	233	40.5	54	341.6	288	228.1	147
11.	308.9	323	213.4	53	74.3	244	43.9	44	349.2	288	228.1	114
12.	314.3	329	218.8	55	76.9	253	46.5	37	364.0	301	242.9	97
13.	320.6	336	225.1	56	84.3	277	53.9	36	380.1	314	259.0	86

The utilization of increasing rates of K fertilizer was also of decreasing tendency. In the case of smaller K doses the higher than 100% relative utilization, i.e. the surplus potassium, came from the exchangeable K content of the soil.

Summary

In a six-year experiment we studied the effects of gradually increasing doses of P and K/25 to 150 and 50 to 300 kg/ha, respectively, in order to establish the optimum ratio of NPK for grassland planted in chernozem soil.

In the experiment the most efficient treatment was 300—25—50 kg/ha NPK, that is, the optimum NPK ratio — 1 : 0.08 : 0.17 — was obtained with the lowest P and K levels, for the reason that the soil of the experiment, in spite of its poor P and Medium K status ensured a good PK supply.

The effect of the 2 N levels and the increasing PK doses on the specific nutritive element contents is clearly demonstrable. The PK fertilization with the given N levels decreased the N content of the grassland and increased the concentrations of P and K.

The N and K output per hectare rose at a rate higher than the increase of yield. The increasing doses of P and K fertilizers were utilized with a decreasing tendency.

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Acta Agronomica Hungarica, 40 (1–2), pp. 87–92 (1991)

RESPONSE OF SAFFLOWER TO DIFFERENT LEVELS OF NITROGEN, PHOSPHORUS AND POTASSIUM

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(Received: 17th October, 1988; accepted: 12th December, 1988)

Two field experiments were conducted to study the response of safflower to different levels of nitrogen, phosphorus and potassium fertilizers during 1985 and 1986 seasons.

Seed yield/ha, 100-seed weight, No. of heads/plant, weight of seeds/head, protein and oil contents of seed were significantly affected by N P K treatments. Highest seed yield/ha was obtained from the combination of (92 kg N + 46 kg P_2O_5 + 25 kg K_2O) followed by (92 kg N + 46 kg P_2O_5). Protein content of seeds increased as nitrogen levels increased, while oil content decreased.

Keywords: safflower, *Carthamus tinctorius* L., P —and K— fertilizers, seed-yield

Introduction

Safflower (*Carthamus tinctorius* L.) has been recognized as a plant of economic importance since the beginning of recorded history, until recent years (Knowles and Davis, 1951). It was confined to the arid and semiarid areas around the margins of the Mediterranean Sea (Tackholm and Drar, 1954). Knowles (1955) suggested that safflower requires at least as much nitrogen as small grains, but more phosphorus. Greenhouse studies on phosphorus, potassium and nitrogen responses revealed that the response to nitrogen was the greatest. Knowles and Miller (1965) reported that on some soils where cereal crops respond to phosphorus, safflower yield has sometimes been economically beneficial from phosphorus fertilizer. Jackson and Kreizinger (1962) speculated that safflower responded to nitrogen application, however, no benefit from phosphorus was observed. Application of phosphorus alone decreased seed yield and crude protein content, and increased the oil percentage of seeds (Werkhoven and Massantini 1966). Phosphorus combined with nitrogen increased the seed yield of safflower (Dathi and Ballal, 1964). In India, Mane (1983) found that yield and oil protein contents increased along with increasing N rates from 0 to 100 kg/ha. Application of 50 kg P_2O_5

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had no effect on yield, oil or protein contents. Armendariz (1984) obtained the highest yield of safflower (1.55 t/ha) with application of 100 kg N and 50 kg P_2O_5, compared with 1.03 t in stands given no N or P.

However, few fertilization experiments were conducted on the response of the components of safflower plant yield; the number of heads per plant, of seeds per head and 100-seed weight, or which of them are important in combination to yield. Ahmed et al. (1985) found that the number of inflorescences per plant, seed yield, 100-seed weight and seed protein content of safflower increased by means of increasing rates of applied nitrogen from g to 60 kg/ha. The highest measures were taken from the 60 kg N + 40 kg P_2O_5/ha treatment. The optimum N fertilizer rate for safflower was found to be quite variable as recorded by different investigators It was 75 kg N/ha (Nasr et al., 1978 in Lebanon), 119 kg N/ha (Nour El-Din et al., 1983 in Egypt) and only 59.8 kg N/ha (Sharma and Verma, 1982 in India).

The objectives of the present study were: (1) To determine of requirements of safflower from the three fertilizer elements either singly or in combination. (2) To study the effects of increasing nitrogen levels on seed yield, yield components and oil and protein content.

Materials and methods

A field study was conducted in the Farm of Faculty of Agric., Alexandria University during the 1985 and 1986 seasons. Ten fertilizer treatments were investigated. One was the check treatment receiving none of the three major fertilizers N, P or K. Three treatments were assigned to the three levels of N, e.g. 46, 92 and 138 kg/ha. These three levels of N were combined with P and/or K to constitute the rest of treatments. They were (46 N + 46 P_2O_5), (46 N + 46 P_2O_5 + 25 K_2O), (92 N + 46 P_2O_5), (92 N + 46 P_2O_5 + 25 K_2O), (138 N + 46 P_2O_5), and (138 N + 46 P_2O_5 + 25 K_2O). Urea 46% N, trible superphosphate 46% P_2O_5 and potassium sulphate 50% K_2O were used. Phosphorus fertilizer was added before planting, while N was given in two application after planting and potassium sulphate was added at 50 days from planting. The experimental design was a randomized complete block with four replications. A local safflower variety was grown. Plot size was 6 rows with 40 cm apart and 4 m long. Twenty plants per plot were randomly harvested to count the number of heads/plant and to weigh the seeds/head. Their yields were added to the seed yield of the inert five square meters harvested from each corresponding plot. The yields of plots were conveyed to yield in kg/ha. The hundred seed weight (g) and oil and protein percentage were measured on randomly selected seed samples of each plot (A. O. A. C., 1980). A statistical analysis was done according to the procedures as outlined by Cochrane and Cox (1968).

Results and discussion

Means of seed yield as affected by different fertilizer treatments (Tables 1 and 2) indicate that 46 kg N/ha with or without P had no effect on seed yield as compared to the control treatment. The treatment combining the same levels of NP in addition to K, however, yielded significantly higher than

the control. Application of 46 kg N/ha had no significant effect on all studied traits except weight of seeds/head. The significant response to increased nitrogen levels started at 92 kg N and no further yield increment was obtained from the higher level. Similar results were obtained by Mane (1983), who found that yields were increased with increasing N rates from 0 to 100 kg/ha.

Yields of the treatments-combination clearly indicate that the yield response was for N as such (Figs 1 and 2). These results confirmed those of

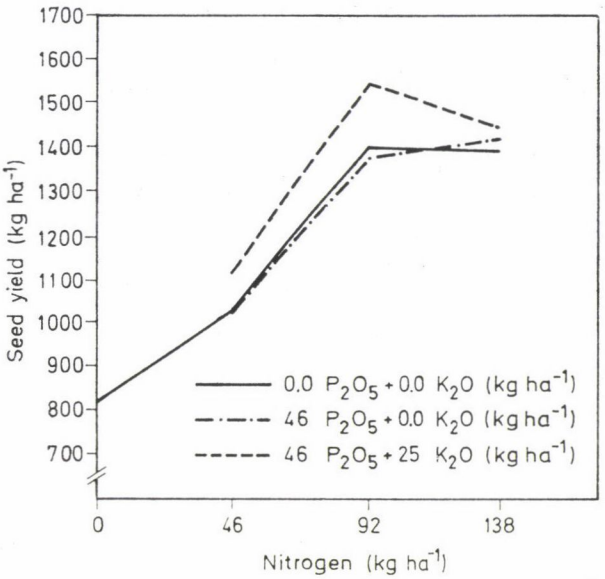

Fig. 1. Effect of nitrogen, phosphorus and potassium fertilizer rates on seed yield of safflower, 1985

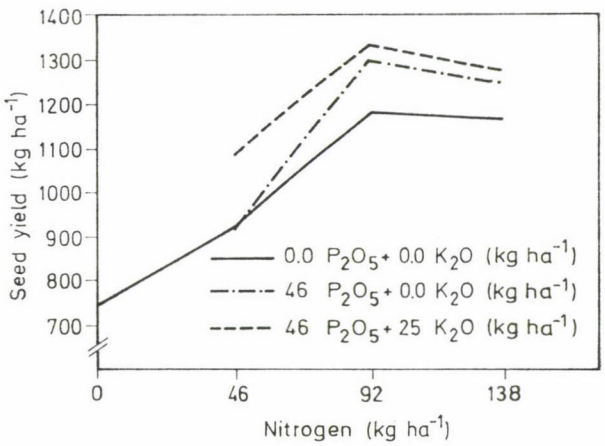

Fig. 2. Effect of nitrogen, phosphorus and potassium fertilizer rates on seed yield of safflower, 1986

Knowles (1955). A considerable yield increase, although not significant was obtained when the treatment combined the best N level along with P and J. e.i. 92 kg N + 46 kg P_2O_5 + 25 kg K_2O. It is of interest to note that this treatment has almost the highest averages of all traits, indicating the importance of a balanced N P K fertilizers application.

Table 1

Seed yield and yield components as affected by nitrogen, phosphorus and potassium fertilizer rates, 1985

No	N P K rates (kg/ha)			Seed yield (kg/ha)	100-seed weight (g)	No. of heads/plant	Weight of seeds/head (g)
1	00	00	00	812 c*	3.99 c	10.02 b	1.33 e
2	46	00	00	1028 c	4.10 c	11.25 b	1.51 d
3	46	46	00	1019 c	4.22 c	11.15 b	1.59 d
4	92	00	00	1400 ab	4.33 bc	13.41 a	1.86 c
5	92	46	00	1380 ab	4.63 ab	14.02 a	2.09 ab
6	46	46	25	1107 bc	4.69 a	14.37 a	1.81 c
7	92	46	25	1530 a	4.73 a	14.63 a	2.16 a
8	138	00	00	1392 ab	4.77 a	13.24 a	1.91 b
9	138	46	00	1416 ab	4.61 ab	14.21 a	1.85 c
10	138	46	25	1439 a	4.46 abc	13.67 a	1.99 abc

* Means followed by the same letter are not significantly different according to Duncan's multiple range test at 5% level

Table 2

Seed yield and yield components as affected by nitrogen, phosphorus and potassium fertilizer rates, 1986

No	N P K rates (kg/ha)			Seed yield (kg/ha)	100-seed weight (g)	No. of heads/plant	Weight of seeds/head (g)
1	00	00	00	738 c*	3.86	9.18 c	1.36 f
2	46	00	00	920 bc	4.00	10.20 ef	1.52 ef
3	46	46	00	916 bc	4.17	10.15 bc	1.60 de
4	92	00	00	1182 a	4.31	11.83 ab	1.72 cde
5	92	46	00	1295 a	4.58	13.15 a	2.10 ab
6	46	46	25	1078 b	4.62	12.96 a	1.62 d
7	92	46	25	1330 a	4.68	13.12 a	2.23 a
8	138	00	00	1160 a	4.70	11.12 abc	1.90 bc
9	138	46	00	1239 a	4.60	12.46 a	1.80 cd
10	138	46	25	1267 a	4.50	12.23 ab	1.86 c

* Means followed by the same letter are not significantly different according to Duncan's multiple range test at 5% level

The yield components for both successive years are given in Tables 1 and 2, respectively. N P K rates were significantly affected the three yield components during the two seasons, except for 100-seed weight in 1986. P combined N significantly increased yield components, especially with N

more than the 46 kg N/ha. These results confirmed those of Dathi and Ballal (1984) and Ahmed et al. (1985), who found that phosphorus combined with nitrogen increased the seed yield and yield components of safflower. The highest values from these traits were obtained by using 92 kg N + 45 kg P_2O_5 + 25 kg K_2O, while the lowest were produced from zero and 46 kg N. with or without phosphorus. These results reflected the responsiveness of safflower to nitrogen fertilizer more than to phosphorus or potassium. Similar results were found by Jackson and Kreizinger (1962) and Mane (1983) has reported that safflower responded to added nitrogen.

Concerning the protein and oil percentages of seed during the 1985 and 1986 seasons (Tables 3 and 4), significant effects for N P K rates were observed on protein content in both seasons, while oil content was significantly affected by N P K in 1985 season only (Table 3). As expected, the highest values of protein contents were obtained from the high levels of nitro-

Table 3

Protein content (%) and oil content of safflower seed as affected by nitrogen, phosphorus and potassium fertilizer rates

No	N P K rates (kg/ha)	Protein content (%)		Oil content (%)	
		1985	1986	1985	1986
1	00 00 00	17.25 b*	16.30 b	33.65 a	32.11
2	46 00 00	17.73 ab	16.52 b	33.32 ab	32.45
3	46 46 00	17.25 b	16.32 b	32.70 bc	31.21
4	92 00 00	18.20 ab	17.70 a	31.04 d	30.62
5	92 46 00	17.92 ab	16.87 ab	32.53 bc	31.73
6	46 46 25	17.50 ab	16.45 b	33.17 ab	32.20
7	92 46 25	17.97 ab	17.31 ab	32.73 bc	31.67
8	138 00 00	18.53 a	17.63 a	32.34 bc	31.42
9	138 46 00	18.00 ab	17.05 ab	32.01 cd	31.26
10	138 46 25	18.20 ab	17.33 ab	31.89 cd	30.91

* Means followed by the same letter are not significantly different according to Duncan's multiple range test at 5% level

gen fertilizer. On the other hand, increasing phosphorus levels did not significantly affect protein content, but the relation was negative between phosphorus and protein content (Werkhoven and Massantini, 1966).

The oil content of safflower seed was negatively related with protein content; accordingly, oil contents significantly decreased along with increasing nitrogen fertilizer levels.

This study of safflower revealed that the maximum seed yield was obtained from the treatment of 92 kg N + 46 kg P_2O_5 + 25 kg K_2O/ha, but the high cost of fertilizer indicates that economically we can fertilize saf-

flower with 92 kg N + 46 kg P_2O_5/ha or 92 kg N/ha only, with no highly significant decrease in yields of seed or protein and oil. Also, safflower selection programs for yield and yield components should be practiced under convenient fertilizer programs in order to solicit a response from the selections.

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Acta Agronomica Hungarica, 40 (1–2), pp. 93—101 (1991)

EFFECT OF OXAMYL ON MACRONUTRIENTS OF THE SOIL AND GROWTH OF TOMATO PLANTS IN PRESENCE OF *MELOIDOGYNE INCOGNITA*

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(Received: 30th January, 1989; accepted: 13th February, 1989)

The effect of oxamyl on plant growth, population and development of root-knot nematode *Meloidogyne incognita* and availability of N, P and K in soil, amended with different nitrogenous fertilizers with superphosphate and potassium sulphate, was studied. The best plant growth was observed at 50 ppm oxamyl/kg soil together with a decrease in the population and the development of root-knot nematode. The availability of N, P and K increased along with an increase in the concentration of oxamyl up to 50 ppm, after 30 days, followed by a decrease.

Keywords: fertilizer, macronutrient, *Meloidogyne incognita*, oxamyl, soil

Introduction

Considerable literature exists on the effect of pesticides on the physico-chemical, biological changes and availability of macro- and micronutrients of the soil (Aldrich and Martin, 1952; Hanson and Nex, 1953; Koike, 1961; Smith, 1963; Singhal et al., 1975; Elliott and Edmunds, 1977; Khasanova et al., 1982; Bayoumi and Waly Taysseer, 1983; Singh and Saxena, 1985 and Singh et al., 1986). However, there is a paucity of information on the effect of the carbamate pesticides on soil properties and availability of nutrients. Therefore, an attempt has been made to determine the effect of oxamyl/methyl N'-N'-dimethyl-N (methylcarbomoyl) oxy-1-thiooxamimidate/, a widely used systemic nematicide on the growth of tomato plants infected with *Meloidogyne incognita* and on the availability of N, P and K, when different nitrogen sources were incorporated with superphosphate and potassium sulphate fertilizers.

Materials and methods

The soil collected at a depth of 0—30 cm from Aligarh Muslim University farm had the following physico-chemical properties: sand = 33.1%; loam = 63.7%; clay = 3.2%; pH = 8.30; EC = 1.25×10^{-4} mmhos/cm; organic matter = 0.21%; CEC = 9.0 m.e./100 g soil; bulk density = 1.07 g/cm^3; real specific gravity = 2.5 g/cm^3 and porosity = 57.

Glazed crocks were filled with one kg/pot of the air-dried, crushed and sieved soil, amended with sodium nitrate, ammonium sulphate, ammonium nitrate and urea separately as nitrogen sources; superphosphate as phosphate and potassium sulphate as potassium, each at the rate of 0.5 g/kg soil respectively. The soil was later treated with oxamyl at the

rate of 5, 10, 50, 100 and 500 ppm/kg soil, separately. Seedlings of tomate (cultivar marglobe), grown in autoclaved soil, were transplanted in each pot and inoculated with 1000 second stage juveniles of *M. incognita*. The pots were then randomly arranged on greenhouse bench. After 60 days the plants were uprooted and the fresh weight and nematode population were determined. The soil was analysed for available N, P and K. Plants grown in nematicides-treated, unfertilized soil and those uninoculated with nematode serve as control.

The isolation of nematodes from soil was done by Oostenbrink's elutriator with Bearman funnel (Southey, 1970) and from roots by varying blender (Stemurding, 1963).

The available N, P and K were determined by methods of proposed by Subbiah and Asija (1956), Olsen et al. (1954) and Jackson (1958) respectively.

Results and discussion

When the soil was amended with different nitrogenous fertilizers as nitrogen sources, and superphosphate as phosphorous, and potassium sulphate as potassium, the highest increase in plant growth was observed at 50 ppm oxamyl per kg in the control as well as in all the amended soils. At 500 ppm, a decrease in plant growth was observed, and the plants exhibited phytotoxicity in the form of marginal leaf scorching. Amongst the fertilizers, maximum growth was recorded with urea, followed by ammonium nitrate, ammonium sulphate and sodium nitrate (Table 1).

The population of root-knot nematode larvae and their development in roots decreased in proportion to the increasing dose of oxamyl, with the highest reduction at 500 ppm in all the treatments (Table 1). When the average reduction in nematode population was considered in fertilized soils, the decrease in nematode population follows this order:

$$\text{Urea} > NH_4NO_3 > (NH_4)_2SO_4 > NaNO_3$$

The high toxicity of urea may be due to the release of ammonium ions during decomposition (Oteifa, 1955). The results are in agreement with Upadhya (1969); Khan (1981) and Pant (1983). The possibility of osmotic pressure generated by ammonia solution (Vassallo, 1967), and the formation of some complexes with certain soil components (Norton, 1978) enhancing the nematode effect, cannot be ignored. An examination of the roots showed nematode gall formation when plants were grown in oxamyl-free soil.

An examination of data presented in table 2 reveals that, immediately after treatment of soil with oxamyl in fertilized soil, the availability of N increased in this order:

$$(NH_4)_2SO_4 > NH_4NO_3 > \text{Urea} > NaNO_3$$

There was no material effect of oxamyl concentration on the availability of N at first, but after 30 days of application of oxamyl, the availability of N increased and thereafter it invariably declined. The maximum increase in available N was recorded up to 50 ppm in all the treatments, followed by a reduction with a further rise in concentration. After 60 days there was

Table 1

Effect of oxamyl on growth of tomato plant and population of M. incognita

Dosage of oxamyl ppm/kg		Increase (+)/decrease (−) over control	
		Population of *M. incognita* (%)	Fresh weight (%)
		Control	
No nematode	0	0.0	0.0
nematode	0	+125.4	0.0
	10	−45.5	+06.7
	50	−58.6	+15.4
	100	−70.9	+02.7
	500	−80.2	+09.5
		$NaNO_3$ + SP + K_2SO_4	
No nematode	0	0.0	0.0
nematode	0	−35.5	−08.15
	10	−62.5	+14.67
	50	−82.1	+26.09
	100	−88.1	+15.27
	500	−97.4	+8.15
		$(NH_4)_2SO_4$ + SP + K_2SO_4	
No nematode	0	0.0	+34.81
nematode	0	−56.5	−09.89
	10	−60.0	+03.85
	50	−70.4	+28.57
	100	−83.7	+20.33
	500	−90.0	−09.89
		NH_4NO_3 + SP + K_2SO_4	
No nematode	0	0.0	+57.03
nematode	0	−51.1	−06.13
	10	−61.1	+04.24
	50	−75.3	+31.13
	100	−87.4	+12.26
	500	−98.0	−11.79
		Urea + SP + K_2SO_4	
No nematode	0	0.0	20.74
nematode	0	−68.5	−02.45
	10	−70.0	+03.68
	50	−79.2	+42.33
	100	−85.6	+19.14
	500	−90.9	−03.68

a general decrease in available N but the availability was higher then the availability at the beginning of all the treatments. The percentage of available N at 50 ppm oxamyl was

$$\text{Urea} > NH_4NO_3 > (NH_4)_2SO_4 > NaNO_3$$

The percent increase of available N with respect to dose after 30 and 60 days was also calculated and is summarized in Table 2. The effect of dose was maximum in control followed by $NaNO_3$, Urea, $(NH_4)_2SO_4$ and NH_4NO_3 after 30 days at 50 ppm dose, while that of the maximum dose after 60 days, the effect was observed in control and minimum urea-amended soils. The percent increase of available N with respect to dose follows the order of 50 ppm $>$ 100 ppm $>$ 10 ppm $>$ 500 ppm in all the treatments, except control.

The increase in available N may be due to an increase in nitrifying bacteria in the soil, as a result of the decrease in nematode population, which might be in part responsible for improve plant growth (Charles and Paul, 1958). Similar results were reported by Singhal and Singh (1974) while studying the effect of nemagon on soil nutrients. Beyond 30 days, however, the effect of oxamyl declined, resulting in a consequent reduction of available N. The decrease in available N after 60 days may also be due in part to the escape of chemically and biologically produced N from the soil into the atmosphere as well as the gradual decomposition of oxamyl into the soil.

Immediately after treatment with oxamyl in fertilized soil, the percentage of available P increased (Table 3). The availability of P wss not materially affected by the concentration of oxamyl at first, but after the soil was amended with $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3 and Urea, the percentage of available P increased up to 50 ppm compared with the unamended soil. After 30 days, the maximum availability of P was observed at 10 ppm in control and at 50 ppm in amended soil. On the basis of percent increase, the maximum P availability was observed in $(NH_4)_2SO_4$ followed by $NaNO_3$, NH_4NO_3 and Urea. After 60 days there was a general decrease in P availability when compared with that at 30 days, but it was higher than that at the beginning. The order of availability with respect to dose follows this order:

10 ppm $>$ 50 ppm $>$ 100 ppm $>$ 500 ppm in control;

50 ppm $>$ 10 ppm $>$ 100 ppm $>$ 500 ppm in $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3 and Urea amended soils.

The increase in P availability may be due to the stimulation in growth and activity of micro-organisms (Alexander, 1961) or could be due to the absorption of oxamyl on soil colloids, resulting in a hindrance to phosphate absorption. These findings agree closely with Winsley (1953) and Martin et al. (1957). Lorenz and Johnson (1953) also observed that more P was released with acid-forming nitrogenous fertilizers. The decrease in P availability at a higher concentration of oxamyl may be due to chemical degradation followed by the bacterial reduction of the available P to the unavailable phosphine.

Table 4 shows that the available K increased in amended soil with maximum in NH_4NO_3 followed by $NaNO_3$, Urea and $(NH_4)_2SO_4$ at zero days. After 30 days of oxamyl application, the percent of K availability increased

Table 2

Effect of oxamyl on the available nitrogen of the soil in presence of different fertilizers at various days

Dosage of oxamyl/kg soil		Available N at various days in mg/kg soil			% increase at various days		% increase and decrease with respect to dose		
		0	30	60	30	60	0	30	60
					Control				
No nematode	0	10.08	11.76	11.20	11.57	11.11		0.00	0.00
nematode	0	10.08	11.76	11.20	11.57	11.11		0.00	0.00
	10	10.08	12.86	12.44	27.58	23.41		9.35	11.07
	50	10.08	13.02	12.74	29.17	26.39	0.0	10.71	13.75
	100	10.08	12.88	12.32	27.78	22.22		9.52	10.55
	500	10.08	12.74	12.04	26.39	19.44		8.33	7.50
					$NaNO_3 + SP + K_2SO_4$				
No nematode	0	12.04	14.26	13.02	18.43	8.14		0.00	0.00
nematode	0	12.04	14.26	13.02	18.43	8.14		0.00	0.00
	10	12.04	15.16	13.16	25.91	9.30	19.44	6.31	1.08
	50	12.04	15.66	13.86	30.07	15.12		9.82	6.45
	100	12.04	15.27	13.72	26.83	13.95		7.08	5.37
	500	12.04	14.98	12.88	24.42	6.98		5.05	−1.07
					$(NH_4)_2SO_4 + SP + K_2SO_4$				
No nematode	0	13.16	16.80	15.26	27.66	15.96		0.00	0.00
nematode	0	13.16	16.80	15.26	27.66	15.96		0.00	0.00
	10	13.16	17.08	15.40	29.78	17.02		2.02	0.92
	50	13.16	17.50	16.24	32.97	23.40	30.55	4.16	6.42
	100	13.16	17.36	15.84	31.91	20.36		3.33	3.80
	500	13.16	16.16	14.42	22.80	9.57		−3.81	−5.50
					$NH_4NO_3 + SP + K_2SO_4$				
No nematode	0	12.43	16.05	13.86	29.12	11.50		0.00	0.00
nematode	0	12.43	16.05	13.86	29.12	11.50		0.00	0.00
	10	12.43	16.24	14.00	30.65	12.63		1.18	1.01
	50	12.43	16.59	15.12	33.47	21.64	23.31	3.36	9.09
	100	12.43	16.38	14.93	31.78	20.11		2.06	7.72
	500	12.43	15.40	13.13	23.89	5.63		−4.05	−5.27
					Urea + SP + K_2SO_4				
No nematode	0	12.32	15.76	14.42	27.92	17.05		0.00	0.00
nematode	0	12.32	15.76	14.42	27.92	17.05		0.00	0.00
	10	12.32	16.24	14.70	31.82	19.32		3.05	1.94
	50	12.32	16.50	15.26	33.92	23.86	22.22	4.69	5.82
	100	12.32	16.27	15.12	32.06	22.72		3.24	4.85
	500	12.32	15.26	14.28	27.92	15.90		−3.17	−0.97

in all the treatments and at 50 ppm it follows the order of $NaNO_3 > (NH_4)_2SO_4 > Urea > NH_4NO_3$ control. The, after 60 days, the K availability declined. The maximum percentage of K availability with respect to dosage was observed in $NaNO_3$ followed by $(NH_4)_2SO_4$, Urea, NH_4NO_3 and control at

Table 3

Effect of oxamyl on the available phosphorus of the soil in presence of different fertilizers at various days

Dosage of oxamyl/kg soil		Available P at various days in mg/kg soil			% Increase at various days		% Increase and decrease with respect to dose		
		0	30	60	30	60	0	30	60
					Control				
No nematode	0	1.32	1.82	1.68	37.88	27.27		0.00	0.00
nematode	0	1.32	1.82	1.68	37.88	27.27		0.00	0.00
	10	1.32	2.50	1.75	89.39	32.58	0.00	37.36	4.17
	50	1.32	2.20	1.95	66.66	47.73		20.87	16.07
	100	1.32	1.98	1.60	50.00	21.21		8.79	−4.74
	500	1.32	1.72	1.50	30.30	13.64		5.49	−10.71
					$NaNO_3$ + SP + K_2SO_4				
No nematode	0	2.05	2.96	2.52	44.39	22.92		0.00	0.00
nematode	0	2.05	2.96	2.52	44.39	22.92		0.00	0.00
	10	2.05	3.26	2.94	59.02	58.04		10.13	16.66
	50	2.05	3.62	3.28	76.58	60.00	55.30	22.29	30.15
	100	2.05	3.04	2.20	48.29	7.31		2.70	−12.69
	500	2.05	2.96	2.20	44.39	7.31		0.00	−12.69
					$(NH_4)_2SO_4$ + SP + K_2SO_4				
No nematode	0	2.53	4.24	3.16	67.58	24.90		0.00	0.00
nematode	0	2.53	4.24	3.16	67.58	24.90		0.00	0.00
	10	2.53	4.64	3.56	83.39	40.71		9.43	12.65
	50	2.53	5.00	4.84	97.62	91.30	91.66	17.92	53.16
	100	2.53	4.16	3.80	64.42	50.19		−1.88	20.25
	500	2.53	2.64	2.24	4.34	−11.46		−37.73	−29.11
					NH_4NO_3 + SP + K_2SO_4				
No nematode	0	2.39	3.38	2.84	41.42	18.82		0.00	0.00
nematode	0	2.39	3.38	2.84	41.42	18.82		0.00	0.00
	10	2.39	3.72	3.00	55.64	25.52		10.05	5.63
	50	2.39	3.97	3.76	64.01	57.32	81.06	15.97	32.39
	100	2.39	2.82	2.54	17.99	6.27		−16.56	−10.56
	500	2.39	2.76	2.24	15.48	−6.27		−18.34	−21.12
					Urea + SP + K_2SO_4				
No nematode	0	2.04	2.88	2.60	41.17	27.45		0.00	0.00
nematode	0	2.04	2.88	2.60	41.17	27.45		0.00	0.00
	10	2.04	3.04	2.80	49.01	37.25		5.55	7.69
	50	2.04	3.24	2.88	58.82	41.17	54.55	12.50	10.76
	100	2.04	2.76	2.38	35.29	16.66		−4.16	−8.46
	500	2.04	2.60	2.26	27.45	10.78		−10.76	−13.07

50 ppm, both at 30 and 60 days; but in urea amended soil this increase was higher than at 60 days. This increase may be due to the solubilization effects caused by certain soil fungi and bacteria which decomposed the alumino-silicate mineral, thus releasing a portion of K contained therein. Some of the

Table 4

Effect of oxamyl on the available potassium of the soil in presence of different fertilizers at various days

Dosage of oxamyl soil	µg/kg	Available K at various days in mg/kg soil			% Increase at various day		% Increase and decrease with respect to dose		
		0	30	60	30	60	0	30	60
					Control				
No nematode	0	49.0	51.0	49.12	4.08	0.24		0.00	0.00
nematode	0	49.0	51.0	49.12	4.08	0.24		0.00	0.00
	10	49.0	53.0	51.0	8.16	4.08		3.92	3.82
	50	49.0	56.0	52.0	14.28	4.08	0.00	9.80	5.86
	100	49.0	51.0	49.12	4.08	0.24		0.00	0.00
	500	49.0	51.0	49.12	4.08	0.24		0.00	0.00
					$NaNO_3 + SP + K_2SO_4$				
No nematode	0	51.42	64.0	52.0	24.46	1.12		0.00	0.00
nematode	0	51.42	64.0	52.0	24.46	1.12		0.00	0.00
	10	51.42	78.0	61.0	51.69	18.63	4.93	21.87	17.30
	50	51.42	104.0	68.0	102.25	32.24		62.50	30.76
	100	51.42	94.0	67.0	82.80	30.29		46.87	28.84
	500	51.42	76.0	62.5	47.80	21.54		18.75	20.19
					$(NH_4)_2SO_4 + SP + K_2SO_4$				
No nematode	0	49.12	64.0	56.0	30.29	14.00		0.00	0.00
nematode	0	49.12	64.0	56.0	30.29	14.00		0.00	0.00
	10	49.12	70.0	56.0	42.50	14.00		9.375	0.00
	50	49.12	92.0	80.0	87.29	62.86	0.24	43.75	42.85
	100	49.12	88.0	78.0	79.15	58.79		27.27	39.28
	500	49.12	78.0	64.0	58.79	9.93		21.87	14.28
					$NH_4NO_3 + SP + K_2SO_4$				
No nematode	0	53.67	71.0	53.0	32.28	−1.24		0.00	0.00
nematode	0	53.67	71.0	53.0	32.28	−1.24		0.00	0.00
	10	53.67	86.0	53.0	60.23	−1.24		22.12	0.00
	50	53.67	90.0	64.0	67.69	19.24	9.53	26.76	20.75
	100	53.67	88.0	63.0	63.96	17.38		23.94	18.86
	500	53.67	76.0	40.0	41.60	−25.47		7.04	−24.52
					Urea + SP + K_2SO_4				
No nematode	0	50.25	64.0	55.0	27.36	8.63		0.00	0.00
nematode	0	50.25	64.0	55.0	27.36	8.63		0.00	0.00
	10	50.25	78.0	57.0	55.22	13.43		21.87	3.63
	50	50.25	87.0	82.0	73.13	63.18	2.48	35.93	49.09
	100	50.25	84.0	62.0	67.16	23.38		31.25	12.72
	500	50.25	61.0	57.0	21.39	13.43		21.39	3.63

K might have been released from the clay minerals by a shift of equilibrium between the soluble and insoluble forms, since micro-organisms are responsible for removing cations from solution. The decrease in availability after 60 days may be due to the gradual loss of activity and disintegration of oxamyl in the soil.

Summary

When soil was amended with different nitrogen sources, namely $NaNO_3$, $(NH_4)_2SO_4$ NH_4NO_3 and Urea incorporated with superphosphate, and potassium sulphate with different, concentrations of oxamyl, the highest increase in plant growth was observed at 50 ppm oxamyl per kg soil in all the treatments. The population of root-knot nematode decreased in proportion to the increasing dosage of nematicide. Healthy growth and root systems could be positively correlated to released nutrients caused by 50 ppm oxamyl/kg soil. These effects were due to the nematicidal action of oxamyl along with the elimination of gall formation from the roots.

Acknowledgement

These studies are financed by University Grants Commission, New Delhi (India)

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 103–110 (1991)

EVALUATION OF RAPE-SEED POTENTIAL AS AFFECTED BY ROW SPACING AND NITROGEN FERTILIZATION

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(Received: 26th January 1989; accepted: 12th April 1990)

The effects of nitrogen fertilizer levels and row spacing on yield, yield components and oil content of rape seed (*Brassica napus* L.) were studied during 1985 and 1986 in the Al-Qassim region of Arabia, using a split-plot design. The main treatments were four nitrogen fertilization levels (85, 131, 177 and 223 kg N/ha) and subplot treatments were three row spacing (20, 30 and 40 cm). The seed yield and the number of fruits/plant were significantly affected by nitrogen and row spacing treatments, while no significant differences were detected for plant height and oil content. The treatment of 177 kg Na/ha with 40 cm row spacing produced the highest seed yield.

Keywords: rape, *Brassica napus* L., seed yield, oil content, soil spacing, nitrogen fertilization

Introduction

The importance of vegetable oil as a human food is quite indisputable. World production was almost three quarters of all oil of biological origin. In the last decade, vegetable oil production has increased by as much as 50% and the contribution of perennial and tropical crops has decreased in favour of annual temperate oil crops. The three major oil-producing crops are now in the order of their importance: soybean, sunflower and oil-seed rape (*Brassica napus* L.). These three crops produced 24 million tons of oil, which was 55% of the total production (Mielke 1980).

Nitrogen fertilizer studies on oil-seed rape have clearly shown that the increase in nitrogen rate raises the yield of seed and oil, but lowers the oil content (Osborne and Batten 1978, Sotomayor 1978, Joarder 1983 and Nordestgaard et al. 1983). The highest seed yield was obtained from the rate of 202 kg N/ha (Ridley 1973) and 187 kg N/ha (Holmes and Ainsley 1975). Trials in Europe indicated that there is little difference in yield between very close rows of 12 cm and broadcasting. Row widths of 18–24 cm and 36–50 cm are commonly used, but where vigorous weed growth is expected when rape seedlings are young, wider rows allow greater ease of access with less danger of plant damage (Weiss 1984). Drilling produced higher seed yields than broadcasting in Canada, the increase being greatest at low seed rates (Clarke et al. 1978). Daniels et al. (1980) reported that this plant has the ability to compensate for low plant population.

This study was conducted to evaluate the rape seed potential, as affected by nitrogen fertilization and row spacing, under Al-Qassim conditions.

Materials and methods

The field experiments were carried out at the Agricultural Experiment Station, College of Agriculture and Veterinary Medicine, King Saud Univ., Al-Qasseem, Kingdom of Saudi Arabia during the 1984/85 and 1985/86 growing season. The soil was a sandy, torrips amments type. The treatments were imposed in a splitplot design with four replications. Main plots consisted of four nitrogen fertilizer levels, i.e. 85, 131, 177 and 223 kg N/ha. Subplots comprised three row spacings of 20, 30 and 40 cm. One-third of each nitrogen level was applied before planting; one-third about 5 weeks after planting, and the remainder about 8 weeks after planting. The source of N used was urea (46% N), the cultivar was Cressor, and each subplot was 3×5 m. Twenty mature plants of each subplot were harvested at random and the following characteristics were measured: plant height (cm), no. of branches/plant and no. of fruits/plant. Twelve square meters were harvested from each subplot and seed yield was calculated in kg/ha. The oil content of seeds (%) was determined on randomly selected seed samples of each subplot, using the *Soxhlet*-method (AOAC 1980). The statistical analyses appropriate to the split-plot design were done according to the procedures outlined by Cochrane and Cox (1968).

Results and discussion

Seed yield

Analyses of variance results of the 1985 and 1986 seasons showed significant effects of nitrogen fertilizer and row spacing, as well as the interaction between nitrogen fertilization and row spacing on seed yield/ha (Tables 1 and 4). The rape seed yield significantly increased with an increasing application of nitrogen fertilizer, up to 177 kg N/ha. Nitrogen rate higher than this produced nitrogen additional seed yield increase during both seasons (Tables 2 and 5 and Figures 1 and 2). Similar results were obtained by Ridley, 1973 and Holmes ans Airsley, 1975.

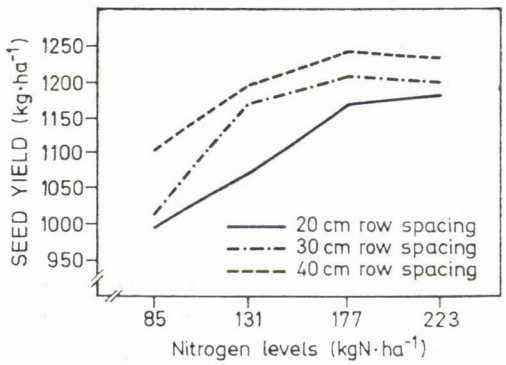

Fig. 1. Influence of nitrogen fertilizer and row spacing on rape-seed yield ($kgha^{-1}$), 1985

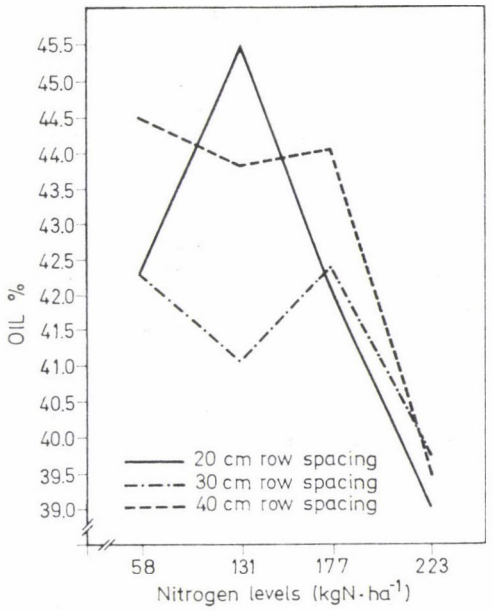

Fig. 2. Influence of nitrogen fertilizer and row spacing on rape-seed yield ($kgha^{-1}$), 1986

With respect to the influence of row spacing on seed yield, significant increases were detected in seed yield and the highest seed yield was obtained using 40 cm row spacing (Tables 2 and 5). This result was probably due to rapeplants in the wider spaces bearing more branches and fruits, and had the ability to compensate for low plant population (Daniels et al. 1980).

Table 1

Analysis of variance summary for the seed yield, yield components, plant height and oil content of rape-seed 1985

Source of variation	df+	Seed yield/ha	No of fruits/plant	No of branches/ plant	Plant height	Oil content
Blocks	3					
Nitrogen (N)	3	**	**	*	NS	*
Error a	9					
Row spacing (S)	2	**	**	**	NS	NS
N × S	6	**	**	NS	NS	NS
Error b	24					

*, ** = Significant at 0.05 and 0.01 level, respectively.
df+ = Degrees of freedom.
NS = Not significant.

Table 2

The effect of nitrogen fertilizer and row spacing on seed yield, yield components, plant height and oil content of rape-seed 1985

Treatments	Seed yield (kg/ha)	No of fruits/plant	No of branches/plant	Plant height (cm)	Oil content (%)
Nitrogen fertilizer (kgN/ha):					
85	1038.93 C*	74.76 C	4.52 C	81.3	44.4 A
131	1141.30 B	84.06 B	5.57 B	83.2	43.10 AB
177	1198.60 A	89.73 A	6.37 A	83.4	41.20 BC
223	1195.30 AB	87.03 AB	6.17 A	83.7	40.40 BC
Row spacing (cm):					
20	1099.8 b	76.4 b	4.36 b	83.4	42.27
30	1146.6 ab	84.16 a	5.81 a	83.0	42.10
40	1184.2 a	90.47 a	6.73 a	82.3	42.55

* Means followed by the same letter are not significantly different by Duncan's multiple range test at p = 0.05.

Referring to the effect of the interaction between nitrogen fertilizer and row spacing on seed yield, obtained data pointed out that the highest seed yield was achieved from 177 and 223 kg N/ha with 40 cm row spacing, followed by 177 kg N/ha with 30 cm row spacing, whereas the lowest seed

Table 3

The effect of combinations of nitrogen fertilizer and row spacing on seed yield, yield components, plant height and oil content of rape-seed. 1985

Nitrogen levels (kgN/ha)	Row spacing (cm)	Seed yield (kg/ha)	No of fruits/plant	No of branches/plant	Plant height (cm)	Oil content (%)
85	20	998.2 f*	69.1 e	3.36	82.2	44.5
	30	1018.5 f	75.5 de	4.80	81.5	43.8
	40	1100.1 e	79.7 cd	5.4	80.4	44.9
131	20	1070.3 e	72.6 cde	4.10	83.7	42.8
	30	1173.6 cd	87.5 abc	5.72	83.3	42.7
	40	1180.0 cd	92.1 ab	6.90	82.6	43.9
177	20	1160.2 d	83.3 bcd	4.81	83.9	41.6
	30	1205.1 abc	89.5 abc	6.60	83.3	41.3
	40	1230.7 a	96.4 a	7.42	83.1	40.9
223	20	1170.5 cd	80.b cd	5.18	84.0	40.2
	30	1189.5 bcd	86.8 abc	6.12	83.9	40.6
	40	1225.9 ab	93.7 a	7.22	83.3	40.5

* Means followed by the same letten are not significantly different by Duncan' s multiple range test at p = 0.05

Table 4

Analysis of variance summary for the seed yield, yield components, plant height and oil content of rape-seed. 1986

Source of variation	df+	Seed yield/ha	No of fruits/plant	No of branches/ plant	Plant height	Oil content
Blocks	3					
Nitrogen (N)	3	**	**	NS	*	NS
Error a	9					
Row spacing (S)	2	**	**	**	*	NS
N × S	6	**	**	NS	NS	NS
Error b	24					

*, ** = Significant at 0.05 and 0.01 level, respectively.
df+ = Degrees of freedom.
NS = Not significant.

yield/ha was produced from 85 kg N/ha with 20 cm row spacing (Tables 3 and 6).

Plant characters

The amount of fruits and braches/plant was significantly affected by nitrogen and row spacing in 1985, but only by row spacing in 1986. The plant height was significantly affected by nitrogen and row spacing only in 1986 (Tables 1 and 4). The greatest production of fruits/plant was obtained from the levels of 177 or 223 kg N/ha, while the least was from the lowest nitrogen

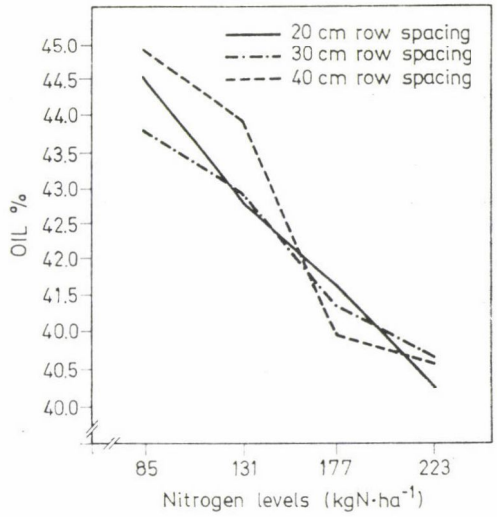

Fig. 3. Effect of nitrogen fertilizer and row spacing on oil per cent of rape-seed, 1985

Table 5

The effect of combination of nitrogen fertilizer and row spacing on seed yield, yield components, plant height and oil content of rape-seed. 1986

Treatments	Seed yield (kg/ha)	No of fruits/plant	No of branches/plant	Plant height (cm)	Oil content (%)
Nitrogen fertilizer (kgN/ha):					
85	912.42 D*	73.81 B	4.06	76.43 B	42.89
131	1050.60 C	77.01 B	4.08	76.66 B	43.43
177	1291.69 A	79.90 AB	4.51	78.33 AB	42.77
223	1199.48 B	86.42 A	4.52	80.30 A	39.35
Row spacing (cm):					
20	995.71 b	71.82 C	3.52 C	80.02 a	42.16
30	1158.73 a	78.33 b	4.31 b	77.30 b	41.36
40	1113.55 a	87.70 a	5.01 a	76.47 b	42.90

* Means followed by the same letter are not significantly different by Duncans's multiple range test at $p = 0.05$.

Table 6

The effect of combinations of nitrogen fertilizer and row spacing on seed yield, yield components, plant height and oil content of rape-seed. 1986

Nitrogen levels (kgN/ha)	Row spacing (cm)	Seed yield (kg/ha)	No of fruits/plant	No of branches/ plant	Plant height (cm)	Oil contet (%)
	20	884.0 f*	66.50 h	3.23	79.5	42.30
85	30	926.8 ef	71.90 g	4.10	75.2	42.27
	40	926.4 ef	83.03 d	4.80	74.6	44.43
	20	997.8 ef	65.47 h	3.20	79.2	45.47
131	30	1203.4 c	79.30 e	4.36	76.8	41.07
	40	950.4 ef	86.27 c	4.66	74.0	43.77
	20	1062.4 de	74.10 f	4.10	79.7	41.93
177	30	1316.2 b	76.43 f	4.30	78.5	42.33
	40	1496.4 a	89.17 b	5.06	76.8	44.03
	20	1038.5 e	81.23 de	3.56	81.7	38.93
223	30	1186.2 cd	85.70 c	4.46	78.7	39.70
	40	1373.6 a	92.33 a	5.53	80.5	39.37

* Means followed by the same letter are not significantly different by Duncan's multiple range test at $p = 0.05$.

level in both seasons. The amount of branches/plant was also affected by nitrogen, similar to that of fruits/plant. The plant height responded positively to nitrogen fertilizer, so that the greatest plant height was obtained from the

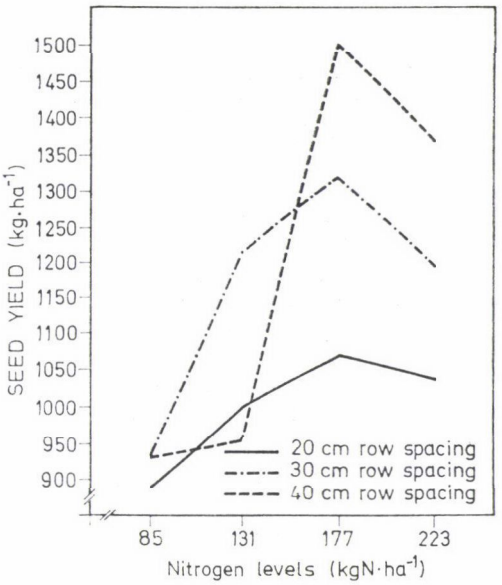

Fig. 4. Effect of nitrogen fertilizer and row spacing on oil per cent of rape-seed, 1986

highest nitrogen level and the shortest plants were produced from the lesser nitrogen levels (Tables 2 and 5). With respect to the influence of raw spacing on the plant characters, significant increases were noted in the number of fruits and branches/plant when row spacing increased, but the plant height significantly decreased with greater row spacing from 20 cm in 1986. As expected the production of fruits/plant was significantly affected by the nitrogen × row spacing interaction in both seasons. Moreover, the highest and lowest values of fruits/plant production were closely related with the highest and lowest values of the seed yield, respectively (Tables 3 and 6).

Oil content

The oil-seed content was significantly affected by nitrogen fertilizer only in 1985 (Table 1). The obtained results of the oil content, as affected by nitrogen fertilizer, revealed the negative relation between nitrogen fertilizer and oil content in both seasons as shown in Tables 2 and 5, and in Figures 3 and 4. The oil content of the seeds lessened as the nitrogen level increased. These results confirm those obtained by Osborne and Batten (1978), Sotomayor (1978), Joarder (1983) and Nordestgaard et al. (1983). Neither row spacing nor row spacing × nitrogen fertilizer interaction significantly affected oil content in both years (Tables 1 and 4).

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 111–116 (1991)

PYRIDOXINE AUGMENTS GROWTH, YIELD AND QUALITY OF MUSTARD THROUGH EFFICIENT UTILIZATION OF SOIL-APPLIED NP-FERTILIZERS

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(Received: 1st August 1989; accepted: 24th February 1990)

Seeds of mustard (*Brassica juncea* Czern. and Coss.) cv. *Varuna* were soaked for 4h in 0 (water), 0.0125, 0.025, 0.05 and 0.1% aqueous pyridoxine solutions and thereafter subjected to 3 combinations of nitrogen and phosphorus ($N_{60}P_{20}$, $N_{90}P_{30}$, $N_{60+30}P_{30}$) for studying the role of pyridoxine in efficient utilization of soil-applied fertilizers. Plants obtained from 0.0125% pyridoxine treated seeds exhibited the highest values for length of main (tap) root, leaf number, dry weight and leaf N, P and K contents at 50, 70 and 90 days after sowing (DAS), net assimilation rate at 50–70 DAS and 70–90 DAS intervals as well as pods/plant, seeds/pod, hecto-litre weight of seeds, seed oil content and yields of seed and oil at harvest. Moreover, the pyridoxine treatment also resulted in "on par" values for above parameters with $N_{60}P_{20}$ and $N_{90}P_{30}$. Thus, the pyridoxine treatment improved the capability of plants to utilize soil-applied NP-fertilizers more efficiently, resulting in 14.3 and 28.4% more yields of seed and seed oil respectively than the water soaked control, together with a net saving of 30 kg N and 10 kg P/ha.

Keywords: *Brassica juncea* Czern. and Coss., pyridoxine, NP-fertilization, leaf N, P and K contents, NAR, seed yield and oil

Introduction

The performance of a crop is a manifestation of various interacting factors. Among such interactions, the soil-root system plays a dominant role in determining productivity. Any impairment in this relationship may result in a poor crop performance (Russell 1970). Thus, it is imperative to evolve such root system of a crop that could efficiently explore soil for water and nutrients. This can be achieved to a large extent by the exogenous application of certain B-vitamin which are responsible for the promotion of root growth (Oertli 1987, Samiullah et al. 1988). For example, increased root growth through pyridoxine (vitamin B_6) applications enhanced plant establishment and augmented the performance of several field crops (Samiullah et al. 1988). Some studies, however, particularly in relation to the interaction of pyridoxine and nutrients, have not been made. By examining interrelationships between the vitamins and nutrients, higher yields may be obtained with a minimal disturbance to the soil ecosystem. Therefore, an attempt was made to study the effect of pre-sowing seed treatment with pyridoxine on the "soil-applied NP-fertilizer use efficiency" of mustard (*Brassica juncea* Czern. and Coss.) cv. *Varuna*.

Materials and methods

Seeds of mustard var. *Varuna* were surface sterilized and soaked for 4h in 0.0125, 0.025, 0.05 and 0.10% aqueous pyridoxine hydrochloride solutions. In addition, two controls (unsoaked and water-soaked) were maintained for comparison. The soaked seeds were sown at the rate of 10 kg/ha in 10 sq.m. plots in a factorial randomized block design. The study was conducted during the 1983/1984 winter season at the Aligarh Muslim University Agricultural Farm, Aligarh, India (27°52′N, 78°51′E and 187 m altitude). The combination of NP-fertilizers comprised $N_{60}P_{20}$ and $N_{90}P_{30}$ (60 and 90 kg N and 20 and 30 kg P/ha). In the third combination ($N_{60+30}P_{30}$), nitrogen was applied as two split doses, i.e. 60 kg N/ha at the time of sowing and 30 kg N/ha at 70 days after sowing (DAS) as top dressing. In this set, basal phosphorus was applied at the rate of 30 kg P/ha. A uniform basal dose of 30 kg K/ha was also applied. Each treatment had three replicates. The sources of nitrogen, phosphorus and potassium were commercial grade urea, monocalcium superphosphate and muriate of potash respectively. The soil of the field was sandy loam showing pH (1 : 2)–8.1, available, N, P and K – 182.8, 22.0 and 268.0 kg/ha respectively. The crop received three irrigations between sowing and harvesting. Weeding was done twice during the entire period of crop growth. 0.01% "Dimecron-100" (Pesticide) obtained from CIBA GEIGY Limited, Bombay, was sprayed to discourage aphid infestation on the crop.

The parameters studied at 50, 70 and 90 DAS included length of the carefully eradicated main (tap) root, leaf number, dry weight and leaf N, P and K contents. Leaf nitrogen and phosphorus contents were estimated according to Linder (1944) and Fiske and Subba Row (1925) respectively. The potassium content in the leaves was determined pyrophotometrically. Net assimilation rates (NAR) were calculated for the period 50–70 DAS and 70–90 DAS (Milthorpe and Moorby 1979). At harvest, pods/plant, seeds/pod, hecto-litre weight of seeds, oil content of seeds, seed yield and oil yield were determined. The data were analysed statistically using the 'F-test' for estimating the significance of, and values for, critical differences (CD). For comparing two treatment means, $P = 0.05$ was also worked out (Panse and Sukhatme 1985).

Results and discussion

Effect of pyridoxine on the performance of mustard

The soaking treatment significantly affected growth parameters, NAR, leaf N, P and K contents, and yield characteristics (Figs. 1–3). However, root length and leaf K at 90 DAS were non-significant. Plants raised from 0.025% pyridoxine-treated seeds exhibited the highest values for all parameters at various stages, except hecto-litre weight which was more in plants obtained from 0.0125% pyridoxine-treated seeds. The effect of this treatment (0.025%) was, however, "on par" with 0.0125% for root length at 70 DAS and dry weight at the three growth stages. Plants obtained from 0.025% pyridoxine-soaked seeds showed a seed oil content increased by 18.0%, seed yield by 14.9% and oil yield by 14.9% and oil yield by 33.2%, in comparison with the water-soaked control.

The results indicate that plants raised from pyridoxine-treated seeds exhibited better growth of their roots (Fig. 1c), which subsequently enhanced their ability to remove more N, P and K from the soil (Figs. 2a-c). The greater supply of these nutrients seems to have promoted assimilatory activities of the treated plants, resulting in high values for various growth parameters (particularly dry weight of the whole plant and NAR) at different growth stages.

These initial advantages gained by plants, as a result of pre-sowing seed treatment with pyridoxine, manifested themselves in the production of more pods and seeds, which in turn enhanced their yield of seeds and oil (Fig. 3b). Samiullah et al. (1988) also reviewed similar beneficial effects of pyridoxine on various field crops.

Effect of NP-fertilizers on the performance of mustard

Among the three selected combinations of nitrogen and phosphorus, $N_{60}P_{20}$ gave the high values for all parameters studied (Figs. 1–3). However, the hecto-litre weight in $N_{60}O_{20}$ decreased, due to the increased number of pods/plant and seeds/pod (Fig. 3a) noted in the $N_{60}P_{20}$, which resulted in

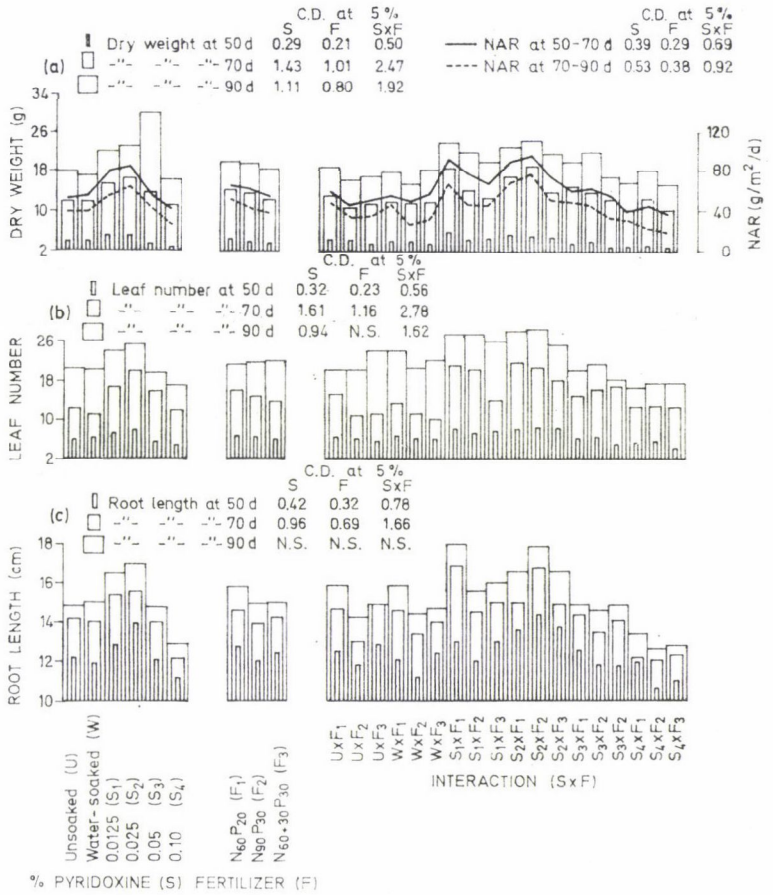

Fig. 1. Effect of pre-sowing seed treatment with aqueous pyridoxine solution (%) and different combinations of N and P on (*a*) dry weight and NAR (*b*) leaf number (*c*) root length of *Brassica juncea* Czern. and Coss. cv. *Varuna*

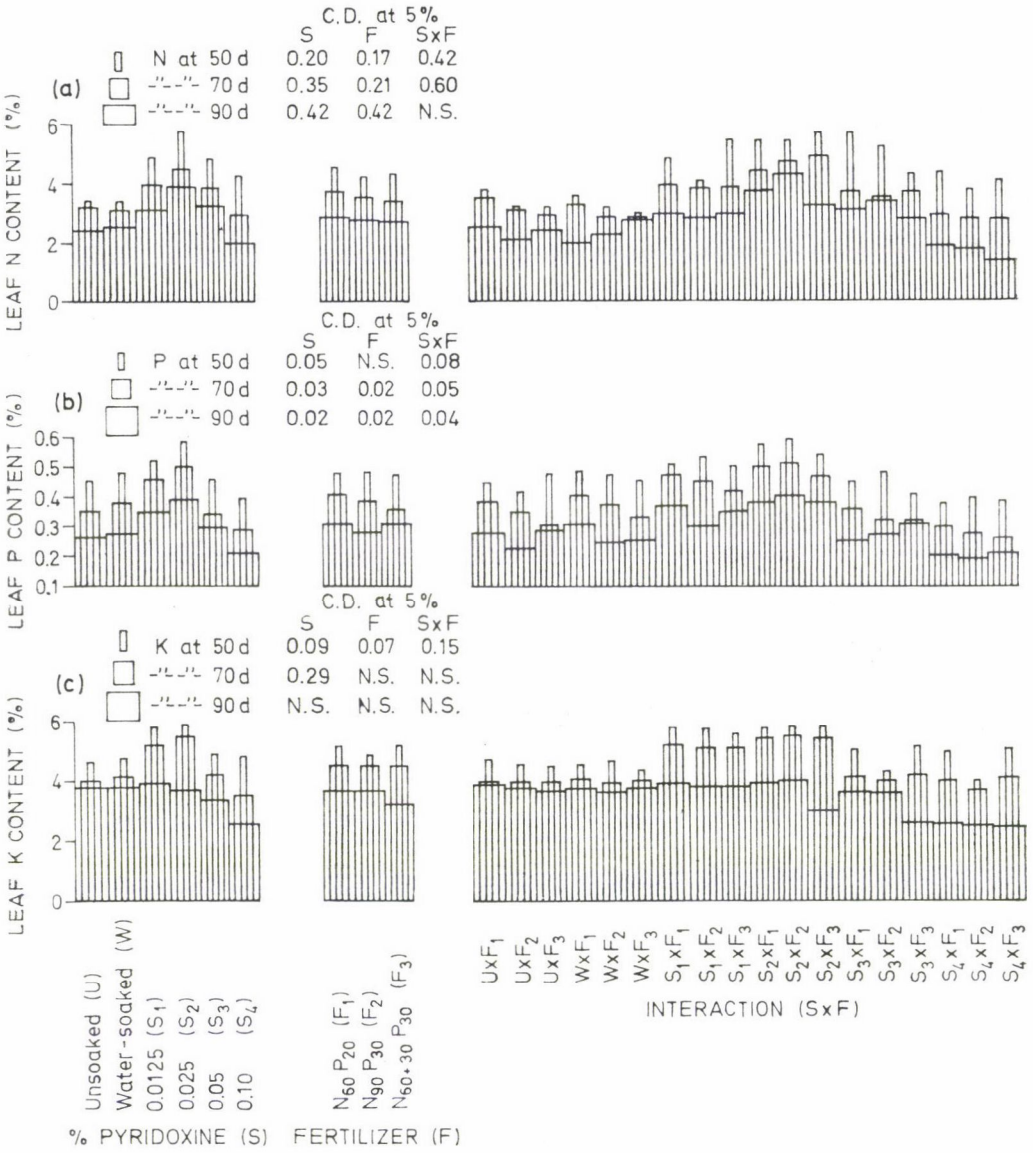

Fig. 2. Effect of pre-sowing seed treatment with aqueous pyridoxine solution (%) and different combinations of N and P on (*a*) leaf N content (*b*) leaf P content (*c*) leaf K content of *Brassica juncea* Czern and Coss. cv. *Varuna*

the distribution of photosynthates in a large number of sinks (seeds), a "dilution effect" phenomenon.

The top dressing of N at 70 DAS proved ineffective, indicating that roots at later growth stages might have either started degeneration or possessed diminished capability for nutrient absorption. According to Fiscus and Mar-

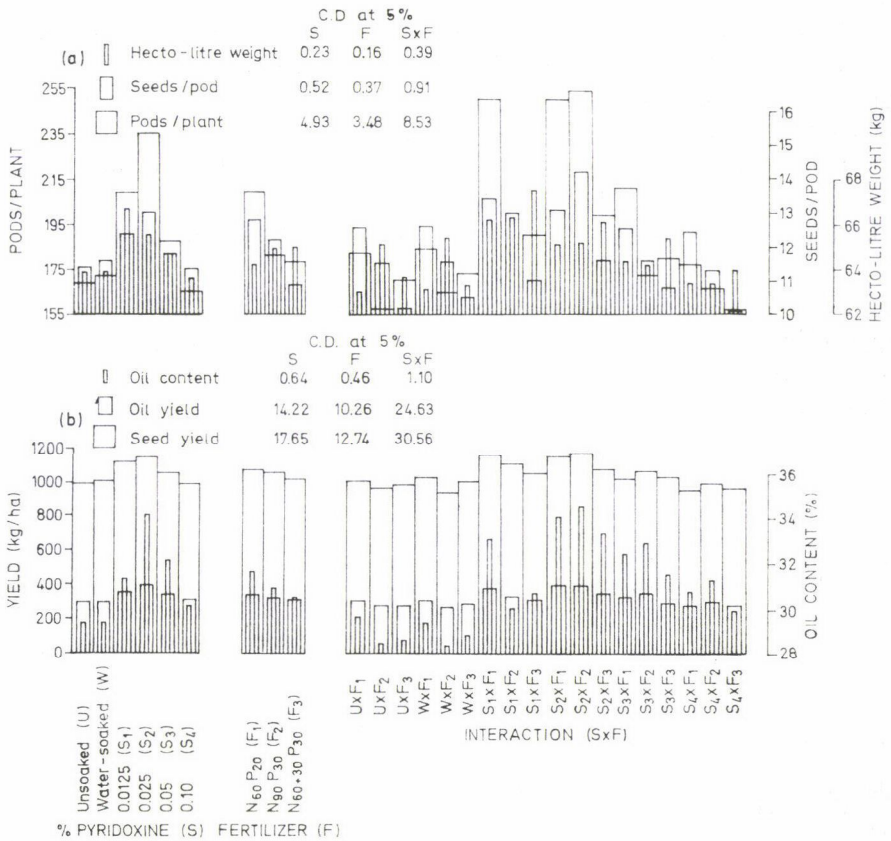

Fig. 3. **Effect of pre-sowing seed treatment with aqueous pyridoxine solution (%) and different combinations of N and P on (*a*) hecto-litre weight, seeds/pod and pods/plant (*b*) oil content, oil yield and seed yield of *Brassica juncea* Czern. and Coss. cv. *Varuna***

khart (1979), absorptive capacity of root systems decreases with age as a result of a decline in hydraulic conductance, due to suberisation of roots at later growth stages.

Interaction between pyridoxine and NP-fertilizers

The interaction of $0.0125 \times N_{60}P_{20}$, in general, exerted a maximum effect on various parameters (Figs. 1–3). However, its effect was "on par" with $0.025 \times N_{60}P_{20}$ and $0.025 \times N_{90}P_{30}$ for several characteristics including root length at 50 DAS leaf number at 70 and 90, DAS dry weight at 70 and 90 DAS, NAR at both intervals, leaf P content at all three growth stages, leaf K content at 50 DAS, pods/plant seed yield and oil yield. Thus, it indicates that early enhancement of root growth by pyridoxine treatment favoured efficient utilization of low dosages of applied fertilizer, i.e., $N_{60}P_{20}$.

It is worth mentioning that plants obtained from 0.0125% pyridoxine-soaked seeds yielded as good results with low fertilizer doses ($N_{60}P_{20}$) as with high fertilizer doses ($N_{90}P_{30}$). Thus, the soaking treatment improved the "soil-applied NP-fertilizer use efficiency" of the plants.

Conclusively, mustard may be grown profitably by soaking the seeds in 0.0125% pyridoxine solution, together with the application of 60 kg N and 20 kg P/ha, achieving 14.3% and 28.4% more seed and oil yield respectively than the water-soaked control receiving 90 kg N and 30 kg P/ha. This results in a net saving of 30 kg N and 10 kg P/ha. Moreover, adoption of this technique, using pyridoxine as a pre-sowing seed treatment, may reduce our dependence on synthetic fertilizers, economize mustard cultivation and curtail soil pollution.

Acknowledgement

The authors (NAK and SAA) are grateful to the University Grants Commission, New Delhi for NET fellowship and Council of Scientific and Industrial Research, New Delhi, for the award of Research Associateship, respectively.

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Acta Agronomica Hungarica, Vol. 40 (1—2), pp. 117—121 (1991)

Plant Genetics and Breeding

EARLY MUTANTS OF PEA (*PISUM SATIVUM* L.) INDUCED BY SOME MUTAGENS

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(Received: 29th June; accepted in revised form: 17th August, 1988)

The effect of some mutagens on the frequency of early mutants was studied.

Treatments with ethyl methane sulphonate and ethylenimine did not result in early mutants. With gamma irradiation early mutants were obtained. X-rays induced, though, early mutants, but their proportion was very small.

The varieties treated differed in the frequency of early mutants.

The number of days from sowing to flowering was lower in the early mutants than in the control. The period between flowering and maturing was much the same with all early mutants as with the control.

In the course of the experiments (1985—1987) 9 viable early mutants were produced, which as an initial material may be of importance in breeding.

Keywords: early mutants, ethylenimine (EI), ethyl methane sulphonate, gamma-rays, pea, various mutagens, X-rays.

Introduction

Among pea mutants valuable for breeding the early mutants deserve special attention.

On the induction of early mutants in cultivated plants, many literary data are available. We want to find an answer to the question of which mutagens are the most suitable to induce early mutants in some pea varieties, so as to use them as initial material in breeding.

Literary review

In recent years a number of publications dealt with studies on early mutants (Gustafsson, 1972, 1979; Gustafsson et al., 1981 in barley; Mikaelsen et al., 1971; Gangadharan et al., 1975; Sajo et al., 1976; Awan et al., 1979; Micke, 1979; Rutger et al., 1980; Rutger, 1981; Sato, 1980; Mingwei et al., 1981 in rice; Ishikawa, 1970; Kwon, 1973 in soybean).

For pea, on the other hand, few literary data are available concerning the results of inducing early mutants.

Early flowering mutants in pea were described by Hoffmann (1959), and Wellensiek (1961, 1965).

Szidorova (1965) treated pea seeds with EMS and obtained several early maturing mutants.

Terescsenko (1966) treated pea plants with various gamma-ray doses. In the progeny there were plants with a vegetation period 3—4—5 days shorter than in the control.

In Poland a new early pea variety (Wasata) was produced by mutagen treatment (in Micke, 1979).

With all this taken into consideration we determined to study the effects of mutagens described in the literature, with special regard to the induction of possible early mutants.

Materials and methods

The experiments were set up in the Laboratory of the Department for Plant Genetics and Breeding and the Szigetcsép trial grounds of the University of Horticulture and Food Industry. The varieties used in the experiments were: Gloria di Quimper, Express, Debreceni világos zöld (round seeded pea), Viridis, Budai gyöngy, Erika (marrowfat pea).

The treatment were:

Chemical treatment

Dry seeds were treated with 0.1-, 0.3- and 0.5% solutions of ethyl methane sulphonate (EMS) and 0.05-, 0.1-, 0.15% solutions of ethylenimine (EI) for 12 hours. The control seeds were kept in distilled water during the treatment.

Physical treatment

Dry seeds were treated with X-rays of 5, 10, 15, 20 kr (1), and gamma rays of 5, 10, 15, 20 kr (2).* The treatments were carried out in the Laboratory of the Isotope Institute of

* (1) and (2): 80 *r*/minute intensity.

the Hungarian Academy of Sciences and the Laboratory of the National "Frédéric Joliot-Curie" Research Institute of Radiobiology and Radiohygienics.

Method of examination

For the second generation (M_2) the seeds were sown with 4 replications per variety and treatment, in random block design, and the plants developing from them were evaluated for the proportion of early mutants.

The number of seeds sown was 600/treatment.

Results

The various mutagen treatments showed great differences in the frequency of early mutants, as seen in Table 1.

Table 1

Frequency of early mutants by treatment in the M_2 generation (1986—1987)

Treatment	Year	Number of plants examined n	Frequency of early mutants	
			n	%
Control	1986	3364	0	0.00
	1987	3232	0	0.00
X-ray	1986	7320	1	0.01
	1987	7091	1	0.01
Gamma-ray	1986	7134	4	0.05
	1987	6991	3	0.04
EI	1986	3895	0	0.00
	1987	3954	0	0.00
EMS	1986	6999	0	0.00
	1987	7009	0	0.00

In 1986 none of the six varieties produced early mutants in response to treatments with EMS and EI.

With 5- and 10kr treatment of gamma-rays, four early mutants were obtained with a vegetation period 6—7 days shorter than in the initial variety. The X-ray treatments produced, though, early mutants, but in a very low percentage (0.01%).

In 1987 a low percentage of early mutants were obtained again in the X-ray treatments. Early mutants appeared in large numbers, on the other hand, when the seeds had been treated with gamma-rays.

Early mutants were only obtained in the varieties Gloria di Quimper, Erika and Viridis; in the varieties Express, Debreceni világos zöld and Budai gyöngy this type of mutant did not occur (Table 2).

Table 2

Frequency of early mutants by variety in the M_2 generation (%)
(1986—1987)

Variety	1986	1987
Gloria di Quimper	0.03	0.03
Express	0.00	0.00
Debreceni világos zöld	0.00	0.00
Viridis	0.02	0.00
Budai gyöngy	0.00	0.00
Erika	0.03	0.03
Total	0.02	0.015

In the early mutants the period between sowing and flowering was shorter than in the control. As regards the number of days from flowering to maturing, there were no essential differences between the early mutants of each variety and the control (Table 3).

Table 3

Detailed data on the frequency of early mutants in the M_2 generation (1986—1987)

Variety	Treatment	Number of mutants n	Sowing-flowering day	Flowering-maturing day	Deviation from the control day
		1986			
Gloria di Quimper	control	0	68	88	—
	gamma-ray 5kr	1	61	81	—7
	gamma-ray 10kr	1	61	82	—6
Viridis	control	0	82	97	—
	X-ray 10kr	1	76	91	—6
Erika	control	0	84	101	—
	gamma-ray 5kr	1	79	95	—6
	gamma-ray 10kr	1	79	94	—7
		1987			
Gloria di Quimper	control	0	67	85	—
	X-ray 5kr	1	60	79	—6
	gamma-ray 10kr	1	60	79	—6
Erika	control	0	82	99	—
	gamma-ray 5kr	1	75	92	—7
	gamma-ray 10kr	1	75	92	—7

Note: 1986: date of sowing: 8 April
total number of plants examined: 25.348

1987: date of sowing: 14 April
total number of plants examined: 25.045

In the course of the experiments (1985—1987) we produced 9 viable early mutants, which as an initial material may be of importance for breeding.

Summary

The effect of various mutagens on the frequency of early mutants was studied.

From the results the following conclusions can be drawn:

(1) Treatments with ethyl methane sulphonate and ethylenimine did not result in early mutants. With gamma irradiation early mutants were obtained. X-ray treatments also produced early mutants, but in very low percentages.

(2) The varieties included in the experiments differed in the frequency of early mutants.

(3) In the early mutants the period between sowing and flowering was shorter than in the control. In the number of days from flowering to maturing, on the other hand, there were not essential differences between the early mutants of any of the varieties examined and the control.

(4) In the course of the experiments (1985—1987) 9 viable early mutants were obtained which as an initial material may be of importance in breeding.

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Acta Agronomica Hungarica, 40 (1–2), pp. 123–127 (1991)

ANALYSIS OF COMBINING ABILITY FOR SEED-OIL CONTENT IN COTTON (*GOSSYPIUM HIRSUTUM* L.)

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(Received 12th April, 1989; accepted 20th June, 1989)

For identifying prospective parents and for formulating breeding procedures most likely to succeed, a combining ability analysis was performed in Cotton (*Gossypium hirsutum* L.), in which 6 Indian lines were crossed with 6 exotic high-oil testers. Variation among parents and crosses and gca effects of lines and testers were highly significant for seed-oil content, seed index and seed-oil index. Sca effects for seed index and seed-oil index were also highly significant. Selections IC 794, T3—11 and the exotic entries Lyman, GM 2 and ORS were adjudged best general combiners. In most crosses, oil content and oil index were higher than those in the best local cultivar SRT 1. The results indicated the need for exploiting both fixable and non-fixable components of genetic variance.

Keywords: combining ability, *Gossypium hirsutum*, oil content, seed-oil index

Introduction

Since the late sixties and the seventies, on a global scale, there have been increasing trends in the consumption and imports of edible oils (Sundaram 1982). The potential of cottonseed as a ready and inexpensive source of edible oil is recognised world-wide. Genetic improvement of cotton, nevertheless, has been mostly due to the fibre quality and yield attributes (Kohel and Lee 1984). Following early reports of genetic variation and response to selection for oil content (Harland 1949), there has been a rather late revival of interest in genetic studies of this trait towards the seventies and eighties (Kohel 1980; Narayanan et al. 1988; Dani and Singh 1989). Thus there is a considerable scope at present for research on the choice of efficient selection methods and genetic populations with improved seed quality (Dani and Kohel 1987, 1989; Dani 1989). The present investigation was undertaken to obtain information on combining ability for seed-oil content in cotton, using the line x tester approach (Kempthorne 1957). The results are reported in this paper.

Materials and methods

From the elite working collection at C. I. C. R., 6 lines were chosen on the basis of comparable maturity duration and yields. These comprised elite cultivars L147, SRT 1, Khandwa 3, selection T3—11 and a reselection from the exotic source IC 794. Six. high-oil lines obtained from Texas, USA (Dani 1984a), were used as tester parents. Stoneville 213

and Tamcot SP 37 are commercial cultivars. ORS is marked with an okra leaf trait and GM 2 has a glandless genotype. In the crop season (June-January) of 1985–86, the 12 entries were grown on the Experimental Farms of the C. I. C. R., Nagpur (21.26° 'N and 79.49° 'E – representing the Central Zone of dryland cotton cultivation) in randomised blocks as 1-row plots of 5 plants (0.60×0.45 m), with 3 repeats. Following anthesis, the 36 crosses were examined in line×tester fashion (Table 2). In the next season, the 12 parents and 36 crosses were grown in a similar field layout. Within each replication, a composite sample of 10 selfed bolls from each cross and from each parent was hand harvested at maturity. Seeds were delnited with commercial sulfuric acid and dried. Oil content was estimated on a wide-line lowresolution Newport NMR Analyser. Weight per 100 seeds was recorded in each sample and the seed index expressed as weight per seed in mg. A seed-oil index giving the weight of oil in mg was derived as follows: seed index×oil percentage/100. Line×tester analysis followed the method of Kempthorne (1967).

Results and discussion

Results of the ANOVA for combining ability and genetic estimates for seed-oil content, seed index and seed-oil index have been presented in Table 1. Parental means and the GCA effects for the 3 traits are given in Table 2. Oil characteristics of 36 crosses and their SCA effects are shown in Table 3.

Table 1

Analysis of variance for treatments and combining ability and other genetic estimates for oil content, seed index and seed-oil index

Source	Df	Oil %	Seed index (mg)	Seed-oil index (mg)
Replications	2	11.91**	2.18	5.72
Treatments	47	7.41**	495.55**	41.33**
Parents	11	2.98*	395.29**	29.72**
Hybrids	35	8.96**	498.0**	44.61**
Parents vs. hybrids	1	1.83	1509.25**	53.75**
Testers	5	24.04**	1977.14**	174.83**
Lines	5	15.77*	286.34	35.03
Lines X Testers	25	4.58**	244.64**	20.33**
Error	94	1.27	9.93	2.20
G.c.a. (Lines)		0.622*	2.32**	0.862*
G.c.a. (Testers)		1.081**	9.63**	8.58**
S.c.a.		1.039	78.96**	5.98**
Due to testers		38.33	56.70	56.00
Due to lines		25.15	8.21	11.47
Due to Lines X Testers		36.53	35.08	32.54

*, ** Significant at 5 and 1% levels of probability

Variance analysis revealed highly significant treatment differences for the 3 attributes, thus suggesting the presence of adequate genetic variation. Performance of the 6 testers appeared to be marginally lower than that reported elsewhere (Kohel 1980; Dani 1984a), which, nevertheless, was higher than the Indian lines, as expected. The differences due to males were highly significant

Table 2

Parental values for oil percentage, seed index, and seed-oil index, and general combining ability effects

Parent	Oil %	G.C.A. effect	Seed index (mg)	G.C.A. effect	Seed-oil index (mg)	G.C.A. effect
Lines						
Khandwa 3	21.48	−0.783	81.80	−3.502	17.58	−1.315
SRT 1	21.33	−0.711	75.46	−7.500	15.90	−2.116
L 147	21.83	−0.039	95.52	−3.649	20.40	−0.315
IC 794	21.02	1.817	119.92	19.616	27.53	5.828
T3-11	21.70	0.911	80.78	3.444	17.68	0.708
Khandwa 2	22.55	−1.194	83.14	−8.408	18.75	−2.791
Testers						
Tamcot SP 37	23.00	−0.850	85.06	−2.496	18.92	−1.097
ORS	24.03	0.311	83.47	1.271	20.06	0.465
Lyman	24.63	1.261	95.26	2.853	24.43	1.591
GM2	22.93	0.494	84.00	4.750	19.28	1.578
Stoneville 213	22.93	0.094	90.44	−6.399	20.75	−1.650
TM 1	22.83	−1.311	90.48	0.022	21.03	−0.887

for all 3 characters, while those due to females were significant in the case of seed-oil content. Such differences, when tested against interaction terms, as is done in a random effect model, may turn out to be non-significant and hence are likely to be under-estimated or not detected (Kaushik et al. 1984).

Combining ability analysis revealed highly significant GCA effects of the testers and significant effects of the lines with respect to all characters, as were also significant SCA effects for seed index and seed-oil index. Considering total variance, the proportional contribution of the testers and that of the lines x testers interaction was relatively higher as compared to that due to the lines. Among the testers, Lyman, GM2 and ORS showed higher GCA effects. The lines IC 794 and T3—11 also showed relatively higher and positive GCA effects. A more or less parallel trend in GCA effects was observed for seed-oil content, seed index and seed-oil index. The oil content among F1's ranged between 19.27% and 25.50% (Table 3). In a large number of crosses, oil content and oil index ranged higher than that in the best local cultivar SRT 1. Most of the superior combinations involved at least one good general combiner. The crosses IC 794×Lyman, Khandwa 2×ORS, L 147×ORS and SRT 1×TM 1 had the highest SCA estimates for oil content. L 147×ORS and IC 794×Lyman showed high SCA effects also for oil index. For an effective evaluation of the seed-oil potential in cotton, the seed-oil index has been recommended as a useful ancillary parameter, for percentages alone are not always reliable (Kohel and Cherry 1983). Unlike oil content and seed index, oil content and oil index in general are positively correlated with each other (Dani 1984a; Dani and Kohel 1987). Crosses having higher values of oil index, unlike many in the case of oil content, did not reflect correspondingly high SCA estimates for this attribute (Table 3).

In the present study, both G.C.A. and S.C.A. effects of seed index and oil index were found to be highly significant, which is consistent with an earlier study (Dani 1984b) involving partial diallel cross analysis. On the basis of large additive genetic components, Kohel and Ramos (1987) inferred that selection for higher oil content should be successful in this crop. Singh et al. (1985) on the other hand, had concluded that the oil content in cotton is under the control of non-additive gene effects. Factors such as intraseasonal variation can result in large G × E interactions, thus imposing restrictions on some genetic models (Dani and Kohel 1987, Ramos (1985) concluded that the generation mean analysis would be adequate to investigate the oil characteristics in cotton, as compared to the diallel. In a recent report (Dani and

Table 3

Performance of crosses and specific combining ability effects for oil percentage, seed index, and seed-oil index

Cross	Oil %	SCA	Seed index (mg)	SCA	Seed-oil index (mg)	SCA
Khandwa 3 X SP 37	20.83	−0.483	70.50	4.785	14.59	−1.781
Khandwa 3 X ORS	23.27	−0.422	78.03	−1.308	18.49	−0.126
Khandwa 3 X Lyman	23.53	−0.728	83.54	−7.091	20.10	−1.535
Khandwa 3 X GM2	22.60	0.617	83.72	2.220	18.61	0.556
Khandwa 3 X S 213	23.50	0.622	80.07	5.609	17.97	0.552
Khandwa 3 X TM 1	19.27	0.394	71.00	5.354	15.04	2.335
SRT 1 X SP 37	20.97	0.789	70.00	−1.042	15.44	0.557
SRT 1 X ORS	23.67	1.117	78.01	2.942	16.80	−0.331
SRT 1 X Lyman	24.90	−1.822	90.22	−9.418	22.62	−3.053
SRT 1 X GM2	21.57	−0.744	70.24	7.091	16.24	1.394
SRT 1 X S 213	19.77	−0.639	57.83	−1.281	11.98	0.294
SRT 1 X TM 1	22.57	1.300	76.51	1.708	16.92	1.140
L 147 X SP 37	21.33	0.106	68.06	2.803	15.84	1.041
L 147 X ORS	21.40	1.400	69.50	13.563	15.88	4.359
L 147 X Lyman	25.43	1.261	87.15	6.647	21.98	1.917
L 147 X GM2	25.17	−1.294	96.12	23.248	24.01	−6.612
L 147 X S 213	23.50	−1.122	72.54	−3.853	17.35	−1.655
L 147 X TM 1	20.63	−0.350	72.52	4.089	15.76	0.950
IC 794 X SP 37	24.53	−0.061	100.64	1.176	24.07	−0.433
IC 794 X ORS	24.33	−1.167	109.28	−8.307	26.47	−2.005
IC 794 X Lyman	24.73	1.761	8.52	13.719	19.59	3.964
IV 794 X GM2	25.50	0.239	106.08	0.411	26.53	0.341
IC 794 X S 213	25.00	0.211	90.51	−4.224	22.58	−0.459
IC 794 X TM 1	24.50	−0.903	118.47	−2.775	28.42	−1.407
T3-11 X SP 37	23.63	1.239	87.86	8.671	18.59	2.155
T3-11 X ORS	23.53	−2.567	84.73	−9.575	20.25	−3.037
T3-11 X Lyman	24.00	0.494	83.74	1.292	19.43	0.535
T3-11 X GM2	24.57	0.139	85.27	−4.003	20.61	−0.378
T3-11 X S 213	24.53	0.578	82.19	3.845	19.40	1.555
T3-11 X TM 1	22.90	0.117	84.67	−0.229	18.31	−0.829
Khandwa 2 X SP 37	21.30	−1.589	75.75	−6.823	17.23	−1.538
Khandwa 2 X ORS	23.37	1.639	75.87	2.684	17.60	1.141
Khandwa 2 X Lyman	22.67	−0.967	79.83	−5.149	18.53	−1.828
Khandwa 2 X GM2	21.27	1.044	74.87	17.529	16.17	4.700
Khandwa 2 X S 213	21.97	0.350	66.26	−0.096	13.51	−0.287
Khandwa 2 X TM 1	19.97	−0.478	64.77	−8.147	12.92	−2.188

Kohel 1989) it has been observed that in the inheritance of oil content and oil index in cotton, dominance effects are more important. In cotton, additive gene effects are preponderant for lint yield and fibre length and strength; while for fibre fineness, dominance effects are considered more important (Meredith 1984). Kaushik et al. (1984) reported higher SCA variances for yield and number of bolls. Meredith (1984) points out that in most genetic studies of cotton, not all basic assumptions are met or the number and quality of genotypes and environments may be deficient.

Acknowledgements

The author is grateful to R. J. Kohel, Laboratory Director, Crop Germplasm Research Unit, USDA, College Station, Texas, USA, for supply of seed and his keen interest. Technical assistance of Mr. G. Gunasekharan is duly acknowledged.

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 129–138 (1991)

STUDIES ON QUANTITATIVE CHARACTERS OF PEA VARIETIES AFFECTED BY ACUTE AND RECURRENT GAMMA IRRADIATION

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(Received: 31th March 1989, accepted: 13th November, 1989)

Gamma irradiation has been long applied to the induction of genetic variations. Relatively high mutation rates can be obtained by the acute and recurrent irradiation of dormant seeds.

The influence of acute and recurrent gamma irradiation was investigated on the quantitative characters of Dukat, Paloma, BR 52, Újmajor early and Marro pea varieties. All five varieties were irradiated with 25, 50, 75 and 100 Gy of gamma rays.

Grains were sown in the experimental field of the Department of Genetics and Plant Breeding. For yield component analysis 30–30 plants were individually observed at every sample. The influence of recurrent irradiation was investigated in two and three subsequent generations.

Varietal differences in radiation sensitivity were observed. The yield components of different varieties changed under similar dose conditions. The Dukat variety was found to be most sensitive to acute irradiation, so the quantitative characters of Dukat may be improved by using this method.

Recurrent irradiation in two subsequent generations was successful in the Paloma variety while treating three subsequent generations proved to be less successful in improving yield components than in two generations.

Keywords: pea, quantitative characters, acute and recurrent irradiation

Introduction

The induction of mutations in plants by ionizing radiation has been carried out on dormant seeds using acute irradiation. The many internal and external factors affecting radiosensitivity and modifying mutagenic effects of radiation on seeds have been extensively investigated by many researchers.

An induced mutation may have value as a germplasm bank for future objectives of plant improvement, even if it is unimportant in present short-term breeding programmes.

Induced mutation may be a principal means of increasing genetic variability.

Guidelines for the practical application of mutation breeding to seed plants have been documented for cereals by Yonezava and Yamagata (1977), and more generally for dicotyledonous plants by Dellaert (1979).

Gamma-ray irradiation has been shown to be highly effective for the induction of mutations in plants. Comparative studies on the effecst acute and chronic doses of gamma rays have been made in terms of growth, survival,

fertility, yield and mutation induction after exposure of both seeds and plants. Acute irradiation of seeds might be more effective in inhibiting growth and decreasing survival and fertility, because of recovery phenomena at low irradiation intensities with chronic irradiation (Fuji 1962, Matsumura 1964). Mabuchi and Matsumura (1964) investigated the frequency of endosperm mutations produced by gamma irradiation in maize pollen. They confirmed that acute irradiation resulted in a higher frequency of mutations than chronic irradiation, but found that the production of mutations was non-linear in acute treatments while linear in chronic ones. It appears that higher mutation rates can be obtained by the acute irradiation of dormant seed than by chronic irradiation of growing plants (Donini and Scarascia–Mugnozza 1968, Nybom et al. 1956, Yamashita 1967, Conger, Constantin and Bottino 1973).

The procedure of recurrent irradiation, or irradiating plant material that has already been irradiated in one or more subsequent generations, has been proposed as a method of accumulating and expanding genetic variation that may be utilized in a breeding programme (Freisleben and Lein, 1943, 1944, Hoffmann and Walther 1961).

Tanaka (1967) concluded that the mutation frequency was increased by recurrent irradiation. Khadr and Frey (1967) reported that oat populations developed by recurrent irradiation with thermal neutrons showed expanded variation in quantitative traits over either the original or the pedigreed populations. Broch and Shaw (1969) concluded that additional variation generated by a recurrent irradiation was greater than that generated by hybridization. Pitirimova (1985) showed that genotypic variability of quantitative characters of barley drastically increased in the M_2 generation. Prohorova and Gladys (1983) stated that repeated irradiation of barley seeds increased the yield in subsequent generations. Mázik and Füredi (1986) concluded that the recurrent neutron irradiation of pea varieties caused further changes to both negative and positive direction in the yield components of pea. These studies suggest that recurrent irradiation could be used for producing types of mutations. These induced mutations are expected to create a new dimension far beyond that achievable using only the available germplasm sources of natural origin.

Materials and methods

The experiments were made with Dukat, Paloma, BR 52, Újmajor early and Marro pea varieties in Gödöllő in 1980–82. Air dry seeds were acutely irradiated with ^{60}Co gamma ray in the gamma-field of the Department of Genetics and Plant Breeding. All five varieties were irradiated with the exposure of 25, 50, 75 and 100 Gy of gamma rays. Grains were sown in seedbeds on the following day of mutagen treatment in the experiment field of the Department.

The plant heights and the yield-determining quantitative characters — such as pod number, seed number/pod, pod length and seed number/plant — were observed. For yield component analysis 30–30 plants were individually measured at every sample and at the

control. Half of the yield of M_1 was sown in the experiment field into randomised block design, in plots of 250 × 125 cm and the planting space of 25 × 5 cm. Statistical analysis of experiments were done by variance analysis. The tables show the average dates of individually observed plants.

The treatment was repeated for accumulating and expanding genetic variation. The other half of the yield of M_1 was irradiated again by the same doses as were used in the previous year. The influence of the recurrent irradiation on the quantitative characters was investigated in two and in three subsequent generations.

Results

Acute irradiations to the air dry seeds

The letality of the seeds of BR 52 and Újmajor early after irradiation with 100 Gy was 100%. The survival rate of Dukat, Paloma and Marro was very low at 100 Gy. A relatively high survival rate was found in M_1 of the BR 52 at 75 Gy of gamma irradiation and it was much higher than at 50 Gy. The other four varieties did not survive this 75 Gy dosage.

The results of the yield component analysis of M_1 generation are shown in Table 1. The pod number of the Dukat variety increased significantly due to the irradiation with 25 and 50 Gy. There were no significant differences among control and treated samples of the other four varieties in this character. The seed number/pod — comparing with the control — decreased as a result of irradiation with 50 Gy at Dukat and Újmajor early. The same result was observed due to the 75 Gy at BR 52 and 25 Gy at Marro. Shorter pods developed at BR 52 (75 Gy dose) and Újmajor early (50 Gy). The pod length of the Dukat increased by irradiating the M_0 with 50 Gy. The 25 Gy of gamma rays increased the number of the seeds of the plants at Dukat, and decreased at BR 52. The yield component analysis of the M_2 generation can be seen in Table 1. The reduced plant heights was registrated at three varieties (BR 52, Újmajor early, Marro). At two varieties there were found significant differences in pod number between the control and the 75 Gy treatment. It was higher at Dukat and lower at BR 52.

The seed number/plant did not vary — it decreased only at BR 52 under the influence of 75 Gy gamma rays. The occurrence of shorter pods was observed at all varieties except Dukat.

The results of the yield determining characters in M_3 indicates (Table 1) that the treatment of M_0 with 50 Gy improved the most yield components (pod number, seed number/plant and plant height) of Dukat variety. In the case of BR 52, the 25 Gy treatment increased almost all of the yield components — two of them (pod number and pod length) significantly. One or two characters was only enlarged or lessened at Újmajor early and Marro varieties. Three of the examined characters decreased due to the 25 Gy dose at Paloma. The 75 Gy of gamma rays increased the plant height and pod number and reduced the length of pods as well as the number of the seeds/plant.

Table 1

Quantitative characters of pea varieties in M_1, M_2 *and* M_3 *generations*

Varieties	Dose Gy	Plant height, cm			Pod number		
		M_1	M_2	M_3	M_1	M_2	M_3
							generation
Dukat	—	—	63.83	64.80	7.23	7.23	4.00
	25	—	60.43	59.40	8.93+	7.20	5.00
	50	—	62.77	72.50+	9.17+	6.90	6.85+++
	75	—	67.57	69.30		9.97++	5.25++
Paloma	—	—	48.13	49.10	6.83	7.47	5.20
	25	—	46.53	39.40---	7.43	7.00	4.90
	50	—	59.50	72.90+++		6.03	6.40+
BR 52	—	—	66.50	66.20	5.47	5.83	4.40
	25	—	63.77	69.70	4.83	5.43	5.20+
	75	—	57.20---	62.85	5.47	4.13--	4.10
Újmajor early	—	—	42.80	41.00	7.97	4.43	5.45
	25	—	39.00---	36.85--	7.77	4.33	5.15
	50	—	39.83--	47.50+++	7.60	5.17	6.00
Marro	—	—	60.90	55.80	6.07	7.33	5.70
	25	—	54.67--	52.30	5.97	7.07	5.40
	50	—	52.77--	49.40---		6.87	5.25

Significant at 5% level + or −
1% level ++ or −−
0.1% level +++ or −−−

Recurrent irradiation to the air dry seeds

Repeated irradiation was carried out with two different exposure doses at Dukat and one-one dose at other varieties because of the seed-shortage of these genotypes.

Recurrent irradiation in two subsequent generations (Table 2) decreased all characters except the pod number at Dukat. This yield component was also higher than at the control at Paloma and Újmajor early varieties. In the case of Paloma there were no significant differences in other characters. At other varieties a decrease of some yield components was established. Subsequent generations of twice-irradiated samples (M'') (Table 2) did not show any deficiency in growth (except the Paloma) and in other yield components. The irradiation with 25 Gy increased the pod number and pod length at BR 52, the plant height at Újmajor early. There was a significant difference between the control and the 50 Gy dose treatment in pod number at Dukat.

Recurrent irradiation in three subsequent generations (Table 2) mostly decreased the analysed yield components. It was concluded that there are

irradiated with acute Co^{60} gamma rays in M_0 generations

Seed number/pod			Pod length cm			Seed number/plant		
M_1	M_2	M_3	M_1	M_2	M_3	M_1	M_2	M_3
3.49	3.49	3.25	5.70	5.76	5.21	25.47	25.47	13.05
3.62	3.30	3.21	5.91	5.52	5.40	31.97+	23.00	16.10
2.60---	3.35	3.57	5.97+	5.69	5.42	24.87	22.50	23.15+
	2.84---	3.12		6.02++	5.48		28.70	16.35
3.59	3.23	4.37	6.16	6.53	6.03	23.97	24.13	23.40
3.67	3.54	3.39---	6.18	6.18--	5.43	27.47	24.78	16.50-
	3.31	2.93---		5.96---	5.24		19.95	16.65-
7.49	6.44	6.23	8.99	8.63	6.25	41.43	37.20	27.75
6.59	6.45	6.03	8.89	9.05	7.22++	31.73	34.27	31.40
4.72---	5.93	6.06	8.42---	8.34--	8.31+++	27.53--	24.80--	26.25
5.08	5.32	4.45	7.58	7.24	6.72	40.47	23.43	23.50
5.40	4.90	4.12	7.57	6.95-	6.37_	41.90	20.93	21.25
4.21---	4.94	4.10	7.18--	7.00	6.67	32.00-	24.50	24.45
3.81	3.17	3.57	6.79	7.16	6.39	22.97	22.83	20.45
3.43-	2.91	3.69	6.35	6.17---	6.31	20.20	21.03	19.35
	2.76-	3.03		6.07---	6.35		18.77	17.15

characters which have changed under the effect of treatment in two subsequent generations, while there are others which could have been influenced only by treatment in three generations.

Results of yield components in M_3 of acute and recurrent treatments can be compared in Table 3. At Dukat variety the repeated irradiation reduced the plant height and seed number/pod, but did not affect the other characters, while acute treatment increased the plant height, the pod number and seed number/plants. The plant height was lessened by all types of treatments with 25 Gy at Paloma. Fewer seeds/pod and seeds/plant were observed from this dosage.

In the case of BR 52 pod number/plant and pod length were increased by acute and recurrent treatments.

The effect of acute and irradiation repeated three times was the same on plant height and pod length of Újmajor early varieties.

The three-repeated irradiation caused changes in plant height and pod length at Marro. Changes in other characters could not be proved statistically.

Table 2

Quantitative characters of pea varieties in M_2'', M_3'' M_3''' generations treated with recurrent

Varieties	Doses Gy M_0, M_1, M_2	Plant height			Pod number		
		M_2''	M_3''	M_3'''	M_2''	M_3''	M_3'''
Dukat	—	63.83	64.80	64.80	7.25	4.00	4.00
	25	59.60	51.30	44.60^{--}	6.77	4.45	3.95
	50	52.70^{---}	60.15	40.15^{---}	6.33	5.15^{+++}	3.85
Paloma	—	48.13	49.10	49.10	5.47	5.20	5.20
	25	49.50	44.35^{--}	40.15	9.60^{+++}	6.30	5.05
BR 52	—	66.50	66.20	66.20	5.83	4.40	4.40
	25	56.50	69.45	52.60^{---}	5.27	5.45^{+}	4.20
Újmajori early	—	42.80	41.00	41.00	4.43	5.45	5.45
	25	41.30	49.45^{+++}	30.55^{---}	5.70^{+}	6.55	4.40
Marro	—	60.90	55.80	55.80	7.33	5.70	5.70
	25	54.23^{--}	58.60	51.75^{-}	6.43	6.05	5.65

M_2'' — treated by gamma rays in two subsequent generations (M_0 and M_1)
M_3'' — treated by gamma rays in two subsequent generations (M_0 and M_1) but not treated in M_2
M_3''' — treated by gamma rays in three subsequent generations (M_0, M_1 and M_2)

Significant at 5% level $^{+}$ or $^{-}$
1% level $^{++}$ or $^{--}$
0.5% level $^{+++}$ or $^{---}$

Discussion

Reactions of Dukat, Paloma, BR 52, Újmajor early and Marro varieties to acute gamma irradiation varied. There were differences among varieties in radiation sensitivity. The same dosage affects the same yield component in different ways at different genotypes. Plant height was the least variable character in the M_1 generation. The 25 Gy dose of gamma rays did not cause any changes at Paloma, Újmajor early and Marro varieties but improved the pod number and seed number/plant at Dukat. The improvement of yield components could only be proved in M_2 at Dukat as a result of the 75 Gy dose. A decrease in plant height (BR 52, Újmajor early, Marro) and pod length (Paloma, BR 52, Újmajor early, Marro) was found (Table 1).

An inheritance of a higher pod number for Dukat (treated with 50 and 75 Gy) was proved in M_3. Positive changes could be registrated at Paloma (50 Gy, plant height, pod number), BR 52 (25 and 50 Gy, pod number and pod length) and Újmajor early (50 Gy, plant height) in our experiments. There

gamma irradiation in two and in three subsequent generations

Seed number/pod			Pod length			Seed number/plant		
M_2''	M_3''	M_3'''	M_2''	M_3''	M_1'''	M_2''	M_3''	M_3''
3.49	3.25	3.25	5.71	5.21	5.21	25.50	13.05	13.05
2.93^{-}	3.49	2.97	4.56^{---}	5.08	5.31	19.23^{---}	15.95	11.40
2.43^{--}	3.18	1.88^{---}	5.29$_{-}$	4.50	5.30	15.23^{---}	16.10	9.13
4.23	4.37	4.39	6.53	6.03	6.03	23.14	23.40	23.40
3.13	3.91	2.83^{--}	5.35	5.20^{---}	5.87	30.33	22.90	12.90^{---}
6.44	6.23	6.23	8.63	6.65	6.65	37.20	27.75	27.75
4.38^{--}	4.62	4.42^{--}	7.43^{---}	7.32^{+}	8.15^{+++}	23.23	28.00	19.05^{-}
5.32	4.45	4.45	7.24	6.72	6.72	23.43	23.50	23.50
2.92^{--}	4.56	2.80^{---}	6.14^{---}	5.79^{---}	6.65	16.63	28.05	16.45
3.17	3.57	3.57	7.16	6.39	6.39	22.83	20.45	20.45
2.68	3.65	3.00	5.98	5.79^{---}	6.25	16.90$^{-=}$	20.75	16.45

were no changes determined in yield components in M_3 at Marro. The most successful acute irradiation was at Dukat, therefore, it can be used for the improving of quantitative characters at this variety.

The repeated irradiation of pea varieties was more successful at Paloma than at the others. This type of treatment had an effect mostly on the seed number/pod, pod length and seed number/plant, but had less influence on the pod number (it was enlarged at Paloma and Újmajor early). The recurrent irradiation in three subsequent generations was less successful in improving the yield components than in two generations.

Acute and recurrent irradiation can be used for widening genetic variation, for increasing mutation frequency and for improving some quantitative characters at different pea varieties.

Our findings confirm the work of Jaranowsky (1976), Monti and Donini (1968), who found similar results examining responses of different varieties for the same acute doses, as well as of Jaranowsky and Micke (1985) who summarized the mutation breeding of pea.

Table 3

Comparison of quantitative characters of pea varieties in M_3 generation

Varieties	Doses Gy M_0, M_1, M_2	Plant height cm			Pod number pc		
		M_3	M_3''	M_3'''	M_3	M_3''	M_3'''
Dukat	—	64.80	64.80	64.80	4.00	4.00	4.00
	25	59.40	51.30	44.60^{---}	5.00	4.45	3.95
	50	72.50^{+}	60.15	40.15^{---}	6.85^{+++}	5.15^{+++}	3.85
	75	69.30			5.25^{++}		
Paloma	—	49.10	49.10	49.10	5.20	5.20	5.20
	25	39.40^{---}	44.35^{--}	40.15	4.90	6.30	5.05
	50	72.90^{+++}			6.40^{+}		
BR 52	—	66.20	66.20	66.20	4.40	4.40	4.40
	25	69.70	69.45	52.60^{---}	5.20^{+}	5.45^{+}	4.20
	75	62.85			4.10		
Újmajor	—	41.00	41.00	41.00	5.45	5.45	5.45
early	25	36.85^{--}	49.45^{+++}	30.55^{---}	5.15	6.55	4.40
	50	47.50^{---}			6.00		
Marro	—	55.80	55.80	55.80	5.70	5.70	5.70
	25	52.30	58.60	51.75^{-}	5.40	6.05	5.65
	50	49.40^{---}			5.25		

M_3 — treated by acute gamma rays in M_0
M_3'' — treated by gamma rays in M_0 and M_1
M_3''' — treated by gamma rays in M_0, M_1 and M_2 generations

Significant at 5% level $^{+}$ or $^{-}$
1% level $^{++}$ or $^{--}$
0.1% level $^{+++}$ or $^{---}$

treated with acute and recurrent gamma irradiation

Seed number/pod pc			Pod length cm			Seed number/plant pc		
M_3	M_3'	M_3''	M_3	M_3'	M_3''	M_3	M_3'	M_3''
3.25	3.25	3.25	5.21	5.21	5.21	13.05	13.05	13.05
3.21	3.49	2.97	5.40	5.31	5.08	16.10	15.95	11.40
3.57	3.18	1.88^{---}	5.42	5.30	4.50	23.15^{+}	16.10	9.13
3.12			5.48			16.35		
4.37	4.37	4.39	6.03	6.03	6.03	23.40	23.40	23.40
3.39^{---}	3.91	2.83^{--}	5.43	5.87	5.20^{---}	16.50^{-}	22.90	12.90
2.93^{---}			5.24			16.65^{-}		
6.23	6.23	6.23	6.25	6.65	6.65	27.75	27.75	27.75
6.03	4.62	4.42^{--}	7.22^{++}	8.15^{+++}	7.32^{+}	31.40	28.00	19.05^{-}
6.06			8.31^{+++}			26.25		
4.45	4.45	4.45	6.72	6.72	6.72	23.50	23.50	23.50
4.12	4.56	2.80^{---}	6.37^{-}	6.65	5.79^{---}	21.25	28.05	16.45
4.10			6.67			24.45		
3.57	3.57	3.57	6.39	6.39	6.39	20.45	20.45	20.45
3.69	3.65	3.00	6.31	6.25	5.79^{---}	19.35	20.75	16.45
3.03			6.35			17.15		

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 139–143 (1991)

DIALLEL ANALYSIS OF YIELDING IN PEPPERS

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(Received: 1st August 1989; accepted: 24th February 1990)

A genetic analysis of the total and commercial yields was carried out on 5 lines and 20 F_1 hybrids of pepper, obtained from complete diallel crossing. The effects of the general and specific combining abilities,and the maternal effects, proved to be significant in the inheritance of both tested features. The general and commercial yields were determined by the nonadditive action of genes much more than the additive one.

Keywords: *Capsicum annuum* L., pepper, total and commercial yields, diallel cross, combining abilities, components of genotype variation

Introduction

Pepper is a plant with high environmental requirements. In the field growing conditions of Poland, the cultivars and breeding lines of this species give very variable yields in different years of growing, which suggests that there occur significant genotype-environment interactions (Karolini 1979, Kowalewski 1983 and 1984, Kordus 1988).

From research carried out in the Department of Horticultural Plant Breeding, of the Chair of Genetics and Plant Breeding in Poznań University of Agriculture, results indicate that heterosis breeding of pepper offers the best chances to obtain early forms with lower environmental requirements and greater yield stability.

To assess breeding value of the initial lines as components for crossing, and to determine possible the maternal effects and importance of the nonadditive action of genes in the inheritance of examined features, the parental forms and the F_1 hybrids obtained from the diallel system according to the method 1 by Griffing (Griffing 1956) were analysed.

Materials and methods

In 1988 five lines of sweet pepper (Table 1) and twenty F_1 hybrids were tested.

The plant material was analysed by a field experiment set in a system of complete randomized blocks in three replications. The results concerning total and commercial yields were worked out statistically using computing program ABS-81 (Dobek et al. 1980).

The percentages of the additive and nonadditive variance, in the general genotype variance of the tested features, were determined according to the scheme given by Smith et al. (1973). Appropriate estimators of variance components were calculated according to the formulae devised by Dobek et al. (1977).

Table 1

Initial lines

Lines	Cultivars	Origin
K-1	Karmin	Hungary
ET 23/399	—	Hungary
T 112/7/23	—	Hungary
PS-206	Poznańska Słodka	Poland
PS-198	Poznańska Słodka	Poland

Results and discussion

Variance analyses of the diallel tables proved a significant role of general effects and specific combining abilities in the inheritance of both features (Table 2). The significance of the *gca* and *sca* effects in shaping the total yield of sweet and hot pepper was found by, among others, Khalf–Allah et al. (1975), Singh and Singh (1978 and 1981), Singh and Rai (1986), Kordus (1988).

Positive general combining abilities in the total and commercial yield were determined for the ET 23/399 line, while significantly negative GCA values were assessed in the two remaining lines of Hungarian origin (Table 3).

Variance analyses also proved the significance of reciprocal effects in the expression of examined features in the F_1 hybrids (Table 2). The important role of the maternal effects in the pepper yield was also found by Silvetti and Grassia (1976), Singh and Singh (1978 and 1981) and Kordus (1988).

Significantly positive ME (maternal effect) values in the total and commercial yield were determined in the ET 23/399 line, while in the T 112/7/23 line significantly negative maternal effects were found in the commercial yield (Table 4).

An estimation of the specific combining abilities established significantly positive values for the both examined features in several combinations of parental pairs, but only in the case of crossing Hungarian lines with the Polish ones (Table 5). The crossing combination PS-206 × PS-198 (the lines originating from the Poznańska Słodka cultivar) was characterized by significantly negative *sca* effects both in the total and commercial yield. This indicates the importance of the genetic distance (differentiation) of the forms used in the crossing project in expression of the *sca* effects among the analysed F_1 hybrids.

Table 2

Variance analysis of diallel tables

Source of variation	df	Mean squares	
		Total yield	Commercial yield
General combining ability	4	0.0178**	0.0173**
Specific combining ability	10	0.0234**	0.0220**
Maternal effects	4	0.0099*	0.0088*
Error	48	0.0036	0.0024

* P = 0.05 ** P = 0.01

Table 3

Estimation of general combining ability effects

Lines	Total yield	Commercial yield
K-1	—0.0394*	—0.0382**
ET 23/399	0.0517**	0.0503**
T 112/7/23	—0.0433*	—0.0454**
PS-206	—0.0011	0.0002
PS-198	0.0321	0.0329

* P = 0.05 ** P = 0.01

Table 4

Estimation of maternal effects

Lines	Total yield	Commercial yield
K—1	0,0040	0,0072
ET 23/399	0.0513**	0.0466**
T 112/7/23	—0.0300	—0.0313*
PS-206	—0.0056	—0.0062
PS-198	—0.0197	—0.0164

* P = 0.05 ** P = 0.01

Table 5

Estimation of specific combining ability effects

Combination of parental pairs	Total yield	Commercial yield
K-1×ET 23/399	0.011	0.004
K-1×T 112/7/23	0.035	0.042
K-1×PS-206	0.074*	0.069*
K-1×PS-198	0.035	0.032
ET 23/399×T 112/7/23	—0.005	—0.013
ET 23/399×PS-206	0.079*	0.082**
ET 23/399×PS-198	0.144**	0.140**
T 112/7/23×PS-206	0.021	0.014
T 112/7/23×PS-198	0.083*	0.089**
PS-206×PS-198	—0.140**	—0.133**

* P = 0.05 ** P = 0.01

Due to the significant role of the reciprocal effects further detailed analyses of the diallel tables proved impossible. In this situation it would appear interesting to determine the proportion of the *gca* (additive) and *sca* (nonadditive) effects in the genotype variance of the examined features.

The results presented in Table 6 indicate that in the toal and commercial yield the nonadditive variance played a much more important role than the additive one. These results agree with those concerning the total pepper yield provided by Khalf–Allah et al. (1975), Singh and Singh (1978) and Singh and Rai (1986).

Table 6

Percentage of additive and nonadditive variance in general genotype variance

Features	Genotype variance	
	additive	nonadditive
Total yield	9.9	90.1
Commercial yield	7.2	92.8

Conclusions

(1) The effects of the general and specific combining abilities played a significant role in the inheritance of the total and commercial yield of the F_1 hybrids.

(2) The effects of the nonadditive action of genes had a great importance in shaping analysed features, which indicates the particular usefulness of the heterosis method in breeding pepper for field growing.

(3) Due to the significance of maternal effects, it appears necessary to perform two directional crossings to find the most valuable idiotypes in the F_1 generation.

(4) Among the tested parental components, the ET 23/399 line having significantly positive general combining abilities and positive maternal effects, both in the total and commercial yield, deserves particular attention.

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 145–150 (1991)

ESTIMATION OF HETEROSIS IN KABULI CHICKPEA[1]

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(Received: 26th September 1989; accepted: 7th December 1989)

To determine heterosis in the Kabuli type chickpea (*Cicer arietinum* L.), 53 F_1S and 47 parents were grown in partially balanced lattice design with 3 replications at Tel Hadya, the main research station of the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria during 1987–88. Fourty-one crosses including 6 at statistically significant level, showed heterosis for seed yield to the extent of 50%. The heterosis in biological yield and harvest index contributed to heterosis in seed yield. The crosses between parents of genetically divergent origin produced greater hybrid vigour than did those between parents of similar origin.

Keywords: cluster, genetic divergence, hybrid vigour, transgressive segregant

Introduction

In chickpea (*Cicer arietinum* L.), a self-pollinated crop, the development of hybrid varieties at commercial scale is not feasible at present because of the problems associated with hybrid seed production. Cytoplasmic male sterility and fertility restorers have not been reported. Muehlbauer and Singh (1987) summarized the reports on hybrid vigour in chickpea, and noted that high vigour had been observed for seed yield and other character.

Much of the information reported on heterosis deals with the desi type chickpea (characterized by angular, small seed size, and coloured seeds, grown mostly in the Indian subcontinent). In the case of Kabuli type chickpea (characterized by ramhead-shaped, large seed size and beige coloured seeds, and grown mostly in the Mediterranean region), information on heterosis is scanty. Furthermore, even in the desi type, heterosis has been reported from a small number of crosses grown at wide spacings. In this study, 53 crosses were grown at normal plant density at Tel Hadya, Syria, to investigate heterosis in the Kabuli type chickpea in a typical Mediterranean climate.

[1] Joint contribution from the International Center for Agricultural Research in the Dry Areas (ICARDA), P.O.Box 5466, Aleppo, Syria and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, P.O., A.P. 502 324 India.

Materials and methods

Forty-seven promising parents, including the elite germplasm and breeding lines, were selected for different characters and crosses were made in 1986–87. The parents and 53 F_1 hybrids were grown during the 1987–88 season at Tel Hadya, the main research of ICARDA, Syria. The sowing was done on 10th December 1987 in a 10×10 partially balanced lattice design with 3 replications. Each entry was sown in a 3 metre single row plot with normal spacing, 45 cm between rows and 10 cm width of row. Five border plants were sown at the ends of each row to avoid border effects. Standard cultural practices were followed as recommended for the winter-sown chickpea crop.

Observations were recorded of the time to flowering and maturity plant height, number of primary and secondary branches, pods/plant, biological yield and seed yield/plot, 100 seed weight, and harvest index. The analysis of variance was carried out for each character separately. Heterosis over better parent and standard check (ILC 482) was estimated for each character in each cross (Liang et. al. 1972). Direct and indirect relationships between heterosis of seed yield and other characters were determined (Steel and Torie 1980). Using the transformed standardized population means, the D^2 value between 2 populations was computed as the summation of squares of differences in mean values for each character. The parents were grouped into clusters based on D^2 values (Mahalanobis 1936).

Results and discussion

Analyses of variance showed significant differences between the entries for all characters except primary branches. Both positive and negative heterosis was observed for all characters when comparison was made with the better parent (Table 1). But when F_1s were compared with the standard cultivar (ILC 482), positive heterosis was observed for days to flower, biological yield, and both positive and negative heterosis for the remaining characters. The mean heterosis for all 53 crosses was positive for plant height, primary branches, pod number, biological yield, and was negative for days to flower, days to maturity, secondary branches, seed-weight, and harvest index (Table 1).

Table 1

Mean and range of heterosis (%) in 53 chickpea crosses over better parent and standard cultivar (ILC 482)

Character	Range of heterosis (%) over				Mean heterosis (%)
	better parent		standard cultivar		
Days to flower	—6.5 to 7.0		0.0 to 7.8		—3.8
Days to maturity	—3.2 to 8.0		—1.1 to 2.3		—1.2
Plant height	—27.8 to 18.6		—13.3 to 48.9		2.1
Primary branches	—25.0 to 66.6		—25.0 to 50.0		7.9
Secondary branches	—58.3 to 42.9		—42.9 to 71.4		—23.7
Pods/plant	—29.7 to 55.3		—23.5 to 73.5		1.4
Biological yield	—31.7 to 45.8		0.7 to 116.9		4.1
Seed yield	—25.7 to 49.5		8.9 to 67.5		11.3
100-seed weight	—28.6 to 8.8		—10.7 to 89.3		—12.3
Harvest index	—31.5 to 13.9		—40.7 to —9.3		—0.1

The heterosis for seed yield was found to the extent of 50%. Six crosses yielded significantly higher than their respective better parents and the standard cultivar (ILC 482) (Table 2). The heterosis reported here was found at the recommended plant populations. Much of the heterosis reported in the literature is based on wide spacing. Therefore, the heterosis obtained in this study, especially in 6 crosses, where it was between 37% and 50%, could be exploited in the cultivar development programme. Production of hybrid varieties in chickpea is not possible at the moment in the absence of many prerequisites, but crosses showing significant heterosis may throw transgressive segregants which might be superior to the standard cultivar.

As far as the authors know, this is the first study on heterosis involving both parents of the Kabuli type. Also, this is the first study on heterosis in the Mediterranean basin comprising southern Europe, North Africa and West Asia.

Table 2

Heterosis (%) in F_1 for crosses which gave significantly more seed yield than better parent and standard cultivar

Crosses	Heterosis (%) over	
	better parent	standard cultivar
FLIP 81—293C × FLIP 83—72C*	45.9	40.8
FLIP 81—293C × FLIP 84—78C*	49.5	33.5
FLIP 81—293C × FLIP 84—93C*	45.7	52.5
ILC 4090* × FLIP 84—91C	38.1	63.6
FLIP 84—164C × ICC 14218*	41.7	67.5
FLIP 85—4C × ICC 14219*	37.8	56.0

*better parent

The heterosis of seed yield had a significant correlation only with the heterosis of biological yield. However, the path coefficient analysis indicated that heterosis for both biological yield and harvest index contributed to the heterosis for seed yield (Table 3). Obviously, biological yield and harvest index are the prime contributors to seed yield. This finding is in contrast with earlier findings, where heterosis in seed yield was found to be influenced by heterosis in the pod number and branch number (Singh et al. 1973, Mandal and Bahl 1984). This difference could be due to earlier researches not including biological yield and harvest index in their studies.

Following D^2 values, 47 parents were grouped in 9 clusters. Twenty-three parents were grouped in in cluster I, whereas cluster VII, VIII, and IX consisted of only one entry each (Table 4). The majority of the breeding (FLIP) lines which were grouped together in different clusters were sister lines derived

Table 3

Direct and indirect relationships between heterosis of seed yield and other characters

	DF	DM	HGT	PRB	SEB	P/P	BYLD	100SW	HI	Correlation coefficient
DF	*0.085*	—0.041	0.002	0.001	—0.010	0.008	0.145	—0.140	—0.015	0.060
DM	0.015	*—0.232*	0.013	—0.005	0.004	0.012	0.075	—0.047	—0.006	—0.171
HGT	0.001	—0.015	*0.207*	—0.009	—0.004	0.007	0.132	—0.078	—0.069	0.171
PRB	0.001	0.012	—0.020	*0.092*	—0.009	—0.057	0.002	0.009	—0.001	0.143
SEB	0.029	0.030	0.030	0.027	*—0.030*	0.076	0.015	—0.056	—0.075	0.137
P/P	0.005	—0.021	0.011	0.039	—0.017	*0.014*	0.073	—0.057	—0.045	0.124
BYLD	0.022	—0.031	0.048	0.001	—0.006	0.017	*0.567*	0.015	—0.246	0.387**
100SW	0.031	—0.034	0.051	—0.003	—0.005	0.024	—0.026	*—0.317*	0.012	—0.267
HI	—0.003	0.004	—0.034	—0.001	0.005	—0.014	—0.330	—0.009	*0.422*	0.040

Residual effect = 0.756, underlined are direct effects.
DF = Days to flower, DM = Days to maturity, HGT = Plant height, PRB = Primary branches, SEB — Secondary branches, P/P = Pods/plant, BYLD = Biological yield, 100SW = 100-seed weight, HI = Harvest index.

from the seme crosses. For example, among the breeding lines in cluster I, FLIP 83–47C, FLIP 83–48C, FLIP 83-72C, FLIP 84–80C, FLIP 84–81C, FLIP 84–92C, FLIP 84–93C, FLIP 84–146C, and FLIP 84–181C were selected from a cross ILC 72 × ILC 215, and FLIP 84–145C had ILC 72 as one of the parents. Some germplasm lines, such as ICC 14197 and ICC 14218, were of similar origin (ex-India). Generally, the F_1 hybrids produced from the cross between the parents from the same cluster group showed non-significant heterosis for seed yield. On the other hand, all of the six crosses (FLIP 81–293C × FLIP 83–72C, FLIP 81–293C × FLIP 84–78C, FLIP 81–293C × FLIP 84–93C, ILC 4090 × × FLIP 84–91C, FLIP 84–164C × ICC 14218, and FLIP 85–4C × ICC 14219) which produced significant heterosis for seed yield involved parents of genetically divergent origin. Therefore, it is suggested that, in order to obtain high hybrid vigour, crosses between parents of genetically divergent origin may be made.

Hybrid cultivars are being increasingly produced in self-pollinated crops. Therefore, it would be worth-while to search for cytoplasmic male sterile and restorer lines and some easy means to cross pollinate them.

Table 4

Clusters for the parents used in 53 crosses

Cluster	Entries
I	— FLIP 82—87C, FLIP 82—189C, FLIP 83—7C, FLIP 83—15C, FLIP 83—47C, FLIP 83—48C, FLIP 83—72C, FLIP 83—98C, FLIP 83—104C, FLIP 84—80C, FLIP 84—81C, FLIP 84—92C, FLIP 84—93C, FLIP 84—102C, FLIP 84—143C, FLIP 84—164C, FLIP 84—181C, FLIP 84—145C, ILC 1929, ILC 1934, ILC 4921, ILC 5342, ILC 493.
II	— FLIP 84—78C, FLIP 85—16C, ILC 3396, ICC 14219.
III	— FLIP 85—1C, FLIP 85—2C, FLIP 85—4C, ILC 4090, ILC 4921, ICC 14197, ICC 14218.
IV	— FLIP 82—150C, FLIP 84—155C, ILC 295, ILC 482, ILC 1919.
V	— FLIP 84—79C, FLIP 84—91C, FLIP 84—99C.
VI	— FLIP 81—293C, ILC 3279.
VII	— FLIP 84—109C.
VIII	— ILC 1920
IX	— FLIP 85—46C

FLIP = (breeding lines developed at ICARDA)

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 151–157 (1991)

Animal Physiology and Biochemistry

COMPOSITION OF THE COLOSTRUM IN TWINNING CATTLE

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(Received: 4th November 1988; accepted: 6th June 1989)

The authors determined the composition of the colostrum in 32 twinning- and 32 single-calf cows on two state farms. The found that the first milked colostrum of cows with Hungaro-Friesian and Holstein-Friesian male parent dropping twins contained significantly more dry matter, total protein, true protein, whey protein, true whey protein and immunoglobulin-G than did that of cows dropping a single calf.

In the other analyses (of casein, NPN, amino acids, biological value, macro- and microelements) no significant difference in the composition of the colostrum could be determined between twinning- and single-calf cows, despite some cases of differences in the averages.

It was established that the sex of the progeny of twinning cows had no influence on the composition of the colostrum.

Keywords: cattle, composition of colostrum, immunoglobulin-G, whey proteins, amino acid composition, biological value, twinning

Introduction

Studying the composition of the colostrum from twinning goats and sheep, we found that their first milked colostrum (0.5–1 hour after dropping) contained significantly more dry matter and total protein on a $P = 0.1–1\%$ level, and more true protein, whey protein, true whey protein and immunoglobulin-G on a $P = 0.1\%$ level than did the colostrum of those dropping a single young. The above differences disappeared 24 hours after dropping, and no significant difference in the composition of the colostrum could afterwards be found between twinning and single-dropping animals. We published these findings in 1988.

Simultaneously with the above investigations, we collected colostra from twinning cows in two places, in the Hajdúnánás State Farm and the Szigetvár State Farm, in order to compare information obtained with cattle to the results of examinations of goats and sheep.

In the course of 1986 and 1987 we collected milk samples from 32 cows, of which 9 dropped 2 bull calves, 8 dropped 2 heifers, and 15 cows birth to calves of mixed sex. In the present paper we wish to present the results of this two-year work.

As reported in an earlier publication (Csapó et al. 1988) we have found neither in the foreign nor in the Hungarian literature any reference to a possible influence of twin dropping on the composition of the colostrum. Since our present work is only aimed at comparing single-calf- and twinning cows for the composition of the colostrum, we do not intend to repeat data on this subject, which is already abundant in the relevant literature. A literary review is therefore not contained in this paper.

Materials and methods

Genotypes examined, colostrum- and milk sampling

Since our previous investigations (Csapó 1984b) unequivocally showed that in the colostra of different genotypes significant breed-dependent differences occurred, we made sure that the cows examined were of the same genotype, and only colostra from single-calf- and twinning cows kept under identical conditions in the same farm were compared. Thus we chose a population with Holstein-Friesian male parent obtained by alternating Holstein-Friesian and Jersey crossing (62,5% Holstein-Friesian + 25% Jersey + 12.5% Hungarian spotted) in the Hajdúnánás State Farm, and a Hungaro-Friesian population of similar genotype in the Szigetvár State Farm. Knowing that the environment may also affect the composition of the colostrum, we compared only the colostra of cows kept under identical conditions.

The population of Holstein-Friesian male parent in Hajdúnánás produced in a traditional bound keeping system, were under summer feeding conditions based decisively on grass. Out of the 17 cows dropping twins, 5 dropped bull calves, 5 heifers, and 7 calves of mixed sex. Eight of the 17 cows examined began the 2nd and 9 the 3rd lactation. Of the 17 control cows simultaneously dropping single calves, 10 began the 2nd and 7 the 3rd lactation.

Of the 15 Hungaro-Friesian cows dropping twins in Szigetvár, 4 gave birth to bulls and 3 to heifers, while 8 cows dropped calves of mixed sex. Both the twinning- and the control cows began the first lactation.

Since in the case of goats and sheep we had found that only colostra extracted immediately after dropping showed differences in composition, we promptly took colostrum samples from cows after dropping (within half- to one hour), and collected no further samples. When taking the samples, we milked some 1.5–2 litres of colostrum by hand.

Chemical analysis of samples

The extracted milk sample was filtered through gauze, then frozen at −20 °C until processed. The dry matter content was determined by drying the samples to steady weight according to standard MSZ 3744-67.

The protein content and protein fractions of the samples were determined with *Kjel-Foss* 16200 type quick nitrogen analyser.

The protein fractions of the milk were separated as follows: from the whole milk fat was expelled in centrifuge in 10 minutes at 8000 r.p.m., then the pH was adjusted to 4.55 by means of a OP-264 type pH meter. The precipitated casein was removed from the whey by centrifuging for 10 minutes at 8000 r.p.m. From the whey (N% × 6.38 = whey protein content) the whey protein was removed after precipitation with 12.5% trichloro-acetic acid, and in the transparent clear solution obtained the nitrogen content (NPN) was determined. By subtracting the NPN from the nitrogen content of the whole milk we obtained the true protein nitrogen content of milk, and by subtracting the NPN from the nitrogen of the whey protein we obtained the true whey protein nitrogen content of the milk. And with the nitrogen content of the whey subtracted from the total nitrogen content the nitrogen content of the casein was obtained. The nitrogen content of the fractions in question multiplied by the conversion factor 6.38 gave the protein content.

The determination of the immunoglobulin-G content of the colostrum was carried out with the simple immuno-diffusion method described by Mancini et al. (1965) in the central

laboratory of our Institute. Our measuring data were checked in the out-patients' department of the Council Hospital of Somogy County. The anti-cattle immunoglobulin-G and the cattle Ig-G standard were obtained from the Gödöllő- and Budapest units of the HUMAN Vaccine Production and Research Institute.

The macro- and microelement contents of colostrum and milk were determined as described by Csapó and Csapó-Kiss (1984a), while the amino acid composition was determined after Moore and Stein (1951) on the basis of descriptions by Csapó and Csapó-Kiss (1986) in their paper. The biological value was calculated on the basis of the amino acid composition by the method of Morup and Olesen (1976).

Results

Dry matter and protein fractions of first-milked colostrum samples collected in the Hajdúnánás State Farm are shown in Table 1, and the analysis results of samples collected in the Szigetvár State Farm in Table 2. In Table 3 the data of the two state farms are summarized; in Table 4 changes in the biological value of colostrum calculated on the basis of amino acid composition, while in Table 5 those in the macro- and microelement content in the case of twin- or single birth are seen.

Table 1

Dry matter content and protein composition of first-milked colostrum from twinning and single-calving cows

Component (g/100 g)	Twinning			Total average	Single-calving
	B + B (n = 5)	H + H (n = 5)	B + H (n = 7)	(n = 17)	n = 17
Dry matter	29.48	29.36	29.80	29.58	24.73
± s	1.97	2.53	1.72	1.93	2.31
Total protein	16.88	18.00	17.16	17.32	14.71
± s	1.45	1.34	1.41	1.39	1.62
True protein	16.45	17.53	16.74	16.89	14.19
± s	1.48	1.37	1.40	1.40	1.44
Whey protein	12.76	13.60	12.71	12.99	10.22
± s	1.37	1.74	0.99	1.32	1.43
True whey protein	12.33	13.15	12.29	12.56	9.71
± s	1.40	1.76	0.99	1.34	1.28
Casein	4.12	4.38	4.44	4.33	4.50
± s	0.27	0.89	0.80	0.69	0.91
NPN × 6.38	0.43	0.45	0.42	0.43	0.52
± s	0.056	0.069	0.067	0.137	0.094
Immunoglobulin-G mg/kg	128.61	132.47	124.73	128.15	104.51
± s	17.12	12.40	12.90	18.81	14.5

An analysis of the data in Tables 1 and 2 reveals that the colostra of cows dropping twins differ in no component from one another significantly. It

seems, thus, that in the case of twin birth the sex of the off-spring does not influence the composition of the colostrum. On the other hand, from the comparison of colostra of twinning- and single-calf cows, the following conclusions can be drawn:

Table 2

Dry matter content and protein composition of first-milked colostrum from twinning and single-calving cows

Component (g/100 g)	B + B (n = 4)	Twinning		Total average Single-calving	
		H + H (n = 3)	B + H (n = 8)	(n = 15)	n = 15
Dry matter	30.45	30.80	30.24	30.41	25.32
± s	1.46	1.44	1.87	1.60	1.24
Total protein	15.80	16.77	16.29	16.25	14.29
± s	0.96	0.92	0.87	0.80	0.60
True protein	15.41	16.36	15.89	15.85	13.81
± s	0.87	0.84	0.87	0.80	0.82
Whey protein	12.18	12.80	12.14	12.28	9.98
± s	0.71	0.76	0.62	0.56	0.31
True whey protein	11.78	12.39	11.74	11.88	9.50
± s	0.69	0.67	0.62	0.57	0.28
Casein	3.63	3.97	4.15	3.97	4.31
± s	0.85	0.55	0.95	0.84	0.83
NPN × 6.38	0.39	0.41	0.40	0.40	0.48
± s	0.082	0.075	0.076	0.099	0.104
Immunoglobulin-G mg/kg	133.12	131.80	138.47	135.71	108.64
± s	16.43	17.17	21.32	22.48	12.18

— The colostrum of cows dropping twins contained 4.85% and 5.09% more dry matter, 2.61% and 1.96% more total protein, 2.70% and 2.04% more true protein, 2.77% and 2.30% more whey protein, 2.85% and 2.38% more true whey protein, 0.17% and 0.34% less casein and 0.09% and 0.08% less NPN × 6.38, respectively, than the colostrum of cows dropping a single calf. In accordance with the whey protein surplus, the colostrum of cows dropping twins contained 23.64 and 27.07 mg/kg more immunoglobulin-G, respectively, than did that of cows with a single calf.

Since the difference in colostrum composition between twinning and single-calf cows was almost the same in the two farms, in Table 3 we summarized the results obtained. (The differences can be read in the table.)

The results of significance analyses of the differences are contained in Table 4. The data of the table show that the differences listed for dry matter, total and true protein, whey and true whey protein and immunoglobulin-G were significant at P = 0.1% level, while those for NPN × 6.38 at P = 1% level.

Table 3

Dry matter content and protein composition of first-milked colostrum from twinning and single calving cows
(summarized data)

Component (g/100 g)	Twinning (n = 32)	Single calving (n = 32)	$\bar{d}$
Dry matter	30.00	25.03	0.97
Total protein	16.79	14.50	2.29
True protein	16.37	14.00	2.37
Whey protein	12.64	10.10	2.54
True whey protein	12.22	9.61	2.61
Casein	4.15	4.41	—0.26
NPN × 6.38	0.42	0.50	—0.08
Immunoglobulin-G (mg/kg)	131.93	106.58	25.35

No significant difference was found in the casein content, and according to our analysis the sex of twins did not influence the colostrum composition either.

According to the data of Table 5 the biological value of the colostrum calculated on the basis of amino acid composition was higher by 9.4 for cows with twins than for those with a single calf both in the Hajdúnánás and Szigetvár State Farm. The higher biological value can be explained by the higher

Table 4

Differences between twinning and single calving cows in the dry matter content and protein composition of first-milked colostrum

Component	1.				2.					
	Hajdúnánás Single calf twins	(n = 17) (n = 17)	Szigetvár Single calf twins	(n = 15) (n = 15)	Szigetvár B+B (n=4) B+H (n=3)		Szigetvár B+B (n=4) B+H (n=8)		Szigetvár H+H (n=3) B+H (n=8)	
Dry matter	9.39	×××	13.77	×××	0.48	Ø	0.24	Ø	0.54	Ø
Total protein	7.13	×××	10.74	×××	2.05	Ø	1.09	Ø	0.94	Ø
True protein	7.83	×××	9.75	×××	2.21	×	1.10	Ø	0.94	Ø
Whey protein	8.30	×××	19.68	×××	1.70	Ø	0.12	Ø	1.75	Ø
True whey protein	8.97	×××	20.53	×××	1.79	Ø	0.13	Ø	1.78	Ø
Casein	0.87	Ø	1.48	Ø	0.91	Ø	1.13	Ø	0.36	Ø
NPN × 6.38	3.15	××	3.05	××	1.72	Ø	0.25	Ø	0.23	Ø
Immunoglobulin-G	5.80	×××	5.79	×××	0.16	Ø	0.55	Ø	0.56	Ø

Ø = P = 10%; × = P = 5%; ×× = P = 1%; ××× = P = 0.1%

whey protein content of the colostrum; namely, it is a well-known fact that the biological value of whey protein is essentially higher than that of casein. The differences in mean value are so small that their reliability cannot be proved by a statistical analysis. There is no difference in the biological value of the colostrum from cows dropping twins of mixed sex.

As seen in Table 6 there is no difference in the macro- and microelement content of the colostrum between twin- and single-calf cows.

To summarize, it can be established that the composition of the colostrum is not influenced by the sex of the offspring. The first milked colostrum of Hungaro-Friesian cows (75% USA–Canadian Holstein and 25% Danish Jersey generation) and of cows with Holstein-Friesian male parent dropping

Table 5

Effect of twin calving on changes in the biological value calculated from the amino acid composition of first-milked colostrum

Biological value of colostrum		Twin-calving				Single calving	$\bar{d}$
		B + B	H + H	B + H	total anim. average		
Hajdúnánás	n	5	5	7	17	17	
State Farm	$\bar{x}$	119.4	121.3	124.4	122.10	112.70	9.40
	±s	12.12	11.04	10.63	12.10	11.40	
Szigetvár	n	4	3	8	15	15	
State Farm	$\bar{x}$	121.9	128.3	126.7	125.70	116.30	9.40
	±s	11.79	12.63	9.88	13.09	9.71	
Farm average		120.5	123.9	125.6	123.80	114.40	9.40

Table 6

Macro- and microelement content in the first milked colostrum of twin- and single calving cows

Component (mg/kg)	Twin calving				Single calving (n = 32)	$\bar{d}$
	B + B (n = 9)	H + H (n = 8)	B + H (n = 15)	Average (n = 32)		
Potassium	2244	2169	2099	2157	1988	169
Sodium	1363	1299	1411	1370	1245	125
Calcium	3054	3196	3062	3093	2963	130
Phosphorus	2455	2526	2314	2407	2117	290
Magnesium	415	429	398	411	382	29
Zinc	31.5	28.2	29.3	29.6	26.4	3.2
Iron	4.96	5.14	5.31	5.17	4.54	0.63
Copper	0.632	0.617	0.587	0.607	0.597	0.01
Manganese	0.131	0.122	0.132	0.129	0.114	0.015

twins contained significantly more dry matter, total protein, true protein, whey protein, true whey protein and immunoglobulin-G than did the first milked colostrum of cows with a single calf. In the other components (casein, amino acids, macro- and microelements) and the biological value of the colostrum, no significant difference between single- and twin birth could be determined in spite of some cases of differences in the averages.

Our final conclusion fully agrees with the one drawn from investigations with goats and sheep. Accordingly, since the immunoglobulin-G is a part of the whey protein, and the whey protein is a part of the total protein, differences in the first-milked colostra of cows are mostly due to the immunoglobulin-G- or whey protein surplus.

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 159–173 (1991)

QUANTITATIVE DETERMINATION OF BACTERIAL PROTEIN FROM THE DIAMINOPIMELIC ACID AND D-ALANINE CONTENT OF RUMEN LIQUOR AND INTESTINES

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(Received: 4th November 1988; accepted: 10th July 1989)

The authors elaborated a method for the determination of diaminopimelic acid (DAPA) and D-alanine (D-Ala) from rumen liquor and intestinal content. By performic acid oxidation used in determining the DAPA — eliminating the disturbing effect of the adjacent amino acids — they could even detect races of it. D-Ala was measured in the form of diastereomer dipeptide by ion exchanging column chromatography.

With the above methods they determined the DAPA- and D-Ala contents of maize silage (0.008; 0.019), lucerne- (0.0006; 0.005) and meadow hay (0.0005; 0.006), as well as of milking concentrate (0.0005; 0.005) and rumen liquor (~0.200; ~0.635) (the first data in the brackets stand for DAPA, the second ones for D-Ala in g/100 g dry matter). They then determined the percentage proportions of DAPA (~0.610) and D-Ala (~2.06) to the total nitrogen from rumen liquor by 5 parallel measurings. These values represent the basis for their further investigations.

Keywords: cattle, rumen liquor, intestinal content, diaminopimelic acid, D-alanine, ion exchanging column chromatography, diastereomer dipeptides

Introduction

In the ruminant species the larger portion of the protein content of feed decomposes in the rumen, and from the ammonia thus produced the microorganisms living in the rumen build up their own proteins, so a considerable proportion of the protein content of feed also transforms into microbial protein. This transformation can be useful when, from the low biological value feed protein or NPN matter, higher biological value bacterium protein is produced. However, in most cases the decomposition of high biological value feed proteins in the rumen is disadvantageous. In the period ahead it will be increasingly important to know what percentage of the feed protein decomposes in the rumen, how much of the amount of protein entering the duodenum comes from the feed, and how much from the bacterium.

In the past years several methods have been elaborated for determining the microbial portion of nitrogen-containing matter passing from the rumen to the abomasum and to the small intestines, respectively. Attempts were made to determine the nucleic acids, and to estimate the microbial origin portion of the nitrogen-containing matter by tracing the B_{12} vitamin and the ^{35}S

isotope. A critical evaluation and summary of these methods was published by Stern and Hoover (1979).

Czerkawski (1974) recently concluded on the protozoa nitrogen by measuring the 2-amino-ethyl-phosphonic acid, and on the bacterial nitrogen content by measuring the 2-6-diaminopimelic acid (DAPA). Namely, 2-amino-ethyl-phosphonic acid decisively occurs in *Protozoa*, while 2-6-diaminopimelic acid (hereinafter DAPA) exclusively in mucopeptides in the cell-walls of bacteria. In spite of the fact that the amount of DAPA in the cell-wall greatly depends on the bacterium species, under steady feeding conditions the proportion of DAPA compared to the total bacterium protein does not change. Therefore, in comparative experiments DAPA can be well employed to estimate the bacterial portion of protein found in the intestinal content.

Schleifer and Kandler (1972) discovered that, besides the diaminopimelic acid, D-Ala occurred only in peptidoglycanes (mucopeptides) present in the cell-walls of bacteria, so this compound can be similarly used to indicate bacterial origin protein and determine its quantity. Knowing the above, Garret et al. (1982) were able to indicate the bacterial origin nitrogen by determining the D-Ala from the rumen liquor. Later Garrett et al. (1987) carried out comparative examinations with diaminopimelic acid and D-Ala in order to find out which of them would be more suitable for the exact determination of bacterial origin nitrogen. They found the D-Ala to be a better indicator of the bacterial nitrogen; that is the variation coefficient of the results obtained with D-Ala was much lower than the one obtained with diaminopimelic acid. Furthermore, the determination of D-Ala was more exact than that of diaminopimelic acid.

Since animal breeders have recently raised increasing demands for the determination of diaminopimelic acid and D-Ala from various materials of biological origin, we have elaborated an ion-exchanging column chromatography method for this purpose. In the present paper a new method of determining these two compounds is described and preliminary results attained by using the method are shown.

Literary review

The determination of DAPA from rumen liquor and intestinal content has been attempted by various methods. Hutton et al. (1971) determined the DAPA with an automatic amino acid analyser, making use of the property of DAPA that, similarly to proline- and unlike the other amino acids, gave off a yellow colour with an acidic ninhydrin solution, the maximum light absorption of which they observed at 420 nm wavelength.

Czerkawski (1974) developed a method for the determination of 2-amino-ethyl-phosphonic acid and DAPA. On determining the latter, he hydrolysed

the protein with acid, purified the hydrolysate on a bone char column, separated the DAPA from the proline on an anion-exchanging column, then determined the DAPA with acidic ninhydrin.

Pongor and Baintner (1980) worked out a simple and quick ion-exchange thin-layer chromatography method combined with video-densitometry for the determination of DAPA, but because of the video-densitometric evaluation the method has not spread in practice.

Edols (1985) determined the DAPA from the hydrolysate of rumen liquor by using a two-column method with an automatic amino acid analyser. With the optimization of the composition of buffers, the DAPA appeared in the chromatogram between methionine and isoleucine, well isolated from them, in the form of a sharp, easily evaluated tip.

Before determining D-Ala from various materials the samples must be prepared, the fractions containing protein concentrated, and the contaminants removed. The purified fraction containing protein is hydrolysed over 22–24 hours at 100–110 °C with 6 mole hydrochloric acid generally used in the amino acid analysis, then at the end of the hydrolysis the hydrochloric acid is concentrated, and the repeatedly concentrated sample is ready for the determination of D- and L-amino acids.

Several methods have been elaborated for the separation and determination of amino acid enantiomers. To study the racemization of pure amino acids, polarimetry was used, then the various enzyme techniques gained ground. These methods have the disadvantage that the D-amino acids in traces cannot be detected by them, and contamination by amino acids coming from the enzymes can be a considerable source of error.

For the separation of D- and L-amino acids, gas chromatography is one of the most rapid methods. The enantiomers can be separated in the form of a diastereomer pair produced with a suitable asymmetrical reagent, or the derivatives made volatile can be separated in an optically active standing phase. The gas chromatographic technique has by now become so precise that the error of determination of enantiomers is lower than 5%, and the reproducibility is extremely high.

For the separation and determination of enantiomers, liquid chromatography has recently gained increasing favour. Weinstein and Weiner (1984) produced the fluorescent derivative 5-dimethyl-aminonaphthaline-1-sulphonyl from amino acids, and with liquid chromatography of inverse phase, applying the N,N'-di-n-propyl-L-alanine and cupric acetate chiral charge, were able to separate the D- and L-enantiomer of the total protein-forming amino acids from a single sample. Marfey (1984) with the help of 1-fluor-2,4-dinitro-phenyl-5-L-alanine amide — which contains a highly reactive fluorine atom — produced diastereomer cupric derivatives separable by liquid chromatography.

To check the optical purity of biologically active substances, various direct methods of liquid chromatography have also been elaborated. The basis of these methods is the chiral column — which consists of a chemically bound L-hydroxy-prolin-Cu^{2+} complex, and the mobile phase, an aqueous solution containing Cu^{2+} ion. With the stationary phase applied, it is possible to check the optical purity of all those compounds which form chelate complexes with the Cu^{2+} ions, such as the amino acids. A deficiency of this method is that the D- and L-forms of only one amino acid at a time can be determined with it.

Manning and Moore (1968) described an ion-exchange column chromatography technique for the separation and quantitative determination of D- and L-amino acids. The method was elaborated primarily to check the stereochemical purity of amino acids used in the course of peptide synthesis, but can be used just as well for the quantitative determination of the D-amino acid occurring in traces beside the L-amino acid. The method is, essentially, the reaction of a L-amino acid N-carboxy-anhydride with the D- and L-amino acids to be examined, in the course of which diastereomer dipeptides are produced, ready for the ion-exchange separation. The dipeptides were produced with the technique described by Hirschmann et al. (1967): the N-carboxy-α-amino acid anhydride was added to the amino acid to be examined in an aqueous medium of 0–2 °C and 10.2–10.4 pH. With a minimum change in the above conditions of reaction they could produce diastereomer diaeptides from the total protein-forming amino acids to about 90%, and thus determine the D- and L-amino acid content. Manning and Moore (1968) using the above method determined 1 part D-amino acid beside 1000 part L-amino acid from samples containing 2 μmol amino acid.

Izumija and Muraoka (1969) worked out a simple method for measuring racemization in the course of peptide synthesis, which essentially consisted of linking the L-Gly-Ala dipeptide with L-leucine under the experimental condition used with the peptide synthesis. In case the experimental conditions do not lead to racemization, the product of racemization will be Gly-L-Ala-L-Leu, while if in the course of the synthesis racemization occurs, then the D-L-isomer produced can be separated from the L-L-derivative in an amino acid analyser or on an ion-exchange thin layer, and quantitatively determined. Since the D-L-isomer is of lower Rf value, of the appearing two peaks (or two spots) the first stands for the L-L-, the second for the D-L-isomer.

Of the methods listed for the determination of DAPA we first attempted the method of Edols (1985), because in our laboratory there are two amino acid analysers. In addition, we hoped to identify all amino acids present in the rumen liquor and intestinal content besides the DAPA. With the precise observance of the parameters described, DAPA could be well separated and evaluated as far as the concentration of DAPA and the total amino acid concentration fell in the same order of magnitude, or as far as the amount of

methionine and isoleucine, the two amino acids beside DAPA, did not exceed the 8–10-fold quantity of DAPA. Then DAPA appeared in the chromatogram as a shoulder peak of methionine or isoleucine, which made evaluation uncertain or even impossible. With the above taken into consideration we introduced a modified new method for the determination of DAPA from rumen liquor or intestinal content. In this paper we describe the modified method for DAPA determination.

What with the literature available and the possibilities of our laboratory, we decided to devise an ion-exchange column chromatography method for separating the D- and L-alanine in the form of diastereomer dipeptide, taking the recent developments in peptide chemistry into consideration. When elaborating the method we ascertained that our experiments as described were reproducible in a laboratory furnished with an amino acid analyser, the method possibly consisting of simple steps and proving suitable for serial examinations. Thus, the method we suggest for the separation and determination of D- and L-alanine is as follows:

— preparation of the sample,
— hydrochloric acid hydrolysis of protein contained in the sample,
— separation of amino acids by ion-exchange column chromatography,
— synthesis of diastereomer alanyl-dipeptides,
— separation and determination of diastereomer alanyl-dipeptides.

Materials and methods

Diaminopimelic acid determination

With a change in the pH and sodium ion concentration of buffers and in the temperature of the ion-exchange column, DAPA can be shifted in the chromatogram within certain limits. The earlier mentioned problem, that the adjacent amino acids present in 8–10-fold quantities suppress the peak of the low concentration DAPA, or DAPA appears as a shoulder peak, arises even when with the changed composition of buffers DAPA is shifted in between methionine and valine. Because of the above the sample to be analysed was subjected to performic acid oxidation (Hirs, 1956) in the course of which cystine was oxidized into cysteic acid, and methionine into methionine sulphone. Cysteic acid appears after the front immediately before aspartic acid, while methionine sulphone between aspartic acid and threonine in the chromatogram, freeing the area between valine and isoleucine. By changing the composition of the buffers we found that DAPA appeared in the place of methionine or somewhat more forward in the chromatogram. As a result of the above changes DAPA appeared between valine and isoleucine in the middle of the chromatogram, and since it was sufficiently far from either the valine or the isoleucine, these two amino acids — though at very high concentrations — did not interfere with the determination of DAPA.

The substances analysed

When elaborating the analysis method we used fish-meal of high methionine- and isoleucine- and 67% crude protein content as a model material. The DL-2,6-diamino-pimelic acid standard was obtained from the Fluka Ag, Buchs SG (catalogue number 21909 1278). When the method was complete we took rumen liquor samples from the rumen of a Holstein-

Friesian cow, and from an aliquot part of it dried by lyophilization performed the DAPA determination. Before the amino acid analysis the crude protein-, true protein- and digestible protein content of the samples was determined with *Kjel-Foss* 16200 (Foss Electric, Denmark) quick nitrogen analyser, using the 6.25 conversion factor.

Hydrolysis, and processing of the hydrolysate

We measured air-dry material of about 10 mg protein content into a surgical ampoule washed with chrome sulphuric acid, and oxidized it with performic acid prepared after Hirs (1956). When the oxidation was completed we immediately cooled the ampoules to −55 °C, and evaporated the material dry in a laboratory lyophilizer (Labor MIM, Hungary, Type OE-950). The residue was hydrolysed in 6 mole hydrochloric acid at 110 °C over 24 hours, as described earlier (Csapó et al., 1986). Processing of the hydrolysate and dilution were carried out as reported in this 1986 publication.

Analysis

The amino acid composition of the samples was determined in an LKB-4101 automatic amino acid analyser (LKB Biochrom Ltd., Great-Britain) using MERCK's amino acid calibration standard. The size of the ion exchange column and the composition of the buffers were:

Ion exchange column: 500×6 mm
Ion exchange resin: CHROMEX UA-8
Buffer flow velocity: 80 cm^3/hour
Ninhydrin flow velocity: 40 cm^3/hour
Column temperature: 50 °C for 60 minutes, then 70 °C up to the end of the analysis
Buffer A: pH = 3.12; Na molarity 0.2; 25 minutes
Buffer B: pH = 4.35; Na molarity 0.2; 60 minutes
Buffer C: pH = 6.35; Na molarity 1.2; 55 minutes
Sodium hydroxide: 0.4 mole; 15 minutes
Equilibration: buffer A; 60 minutes

The chromatograms were evaluated by comparison to areas below the peak obtained at the calibration standard. The standard deviation of results obtained by parallel analyses was calculated with a HT PTK-1050 type pocket computer (Telecommunication Co-operative, Hungary).

D-Ala determination
The substances used

The most essential point of this method is the synthesis and separation of the alanyl-diastereomer dipeptides. Since in peptide syntheses carried out in homogeneous solutions active ester condensation is even today one of the most common methods, because the reaction is almost quantitative and the purification of the product is simple, we employed this method, too. Considering that the separation of alanine from the other amino acids as well as the separation of diastereomer alanyl-dipeptides take place in an aqueous medium, we chose N-hydroxy-succinimide ester (ONSu) from among the active esters, since these esters also excellently bind in an aqueous medium and the active ester by-products that arise in the course of binding does not interfere with the amino acid analysis. As the next step we had to decide what group to select in order to protect the amino group of the acylizing amino acid during the active ester condensation. Since on determining the L–L or L–D peptides with the amino acid analyser, the protective group must be removed — in order to make the compound to be measured nynhydrin positive —, we chose the terciary-butyl-oxy-carbonyl group (BOC), because not only is it easy to build, but also after the dipeptide synthesis the protective group is easy to split with trifluoro-acetic acid or 1 mole acidum aceticum hydrochloric acid.

After choosing the protective group and the active ester we had to decide which of the protein amino acids available would be the best acylizing amino acid. As it is necessary that the acylizing amino acid possesses an asymmetrical centre, and binding takes the shortest possible time, we chose alanine (Ala). The alanyl-alanine dipeptide appears in the chromatogram after the Ala, so separation takes at least 1–1.5 hours. Thus, we tried to find a possibility

for the synthetized diastereomer alanyl-dipeptide to appear in a short time in the chromatogram. Therefore, we chose cystine (CySS) as a second acylizing amino acid, hoping that when oxidizing the tripeptide produced by active ester condensation with performic acid we would obtain 2 dipeptides, one of which, the cysteic acid, could accelerate the elution of dipeptide. In this way the separation of the 2-sulfonic acid-alanyl-alanine dipeptides takes a substantially shorter time.

To this end we synthetized the tertiary-butyl-oxy-carbonyl-L-alanine-N-hydroxy-succinimide-ester (t-BOC-L-Ala-ONSu) and the bis-tertiary-butyl-oxy-carbonyl-L-cystine-bis-N--hydroxy-succinimide-ester (t-BOC)$_2$-L-CySS-(ONSu)$_2$ in the hope that the diastereomer dipeptides obtained would well separate from each other, and the quantitative evaluation would not encounter difficulties. In the second case we should have liked to elaborate a quick method in order to increase the productivity of the procedure by reducing the elution time of D-alanine. The protected active esters of the two amino acids were synthetized in the way described in handbooks on peptide chemistry (Bajusz, 1980).

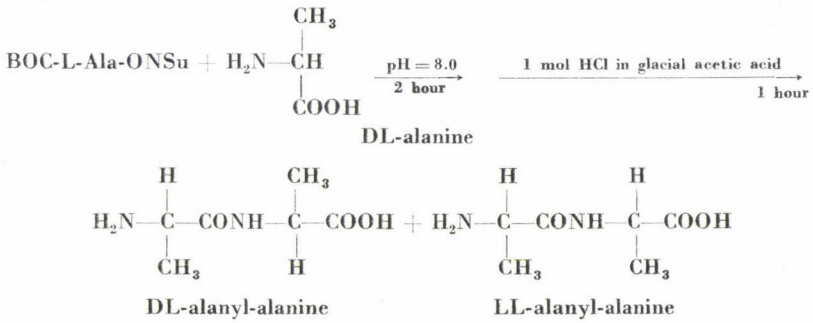
After the synthesis of the active esters, the diastereomer dipeptides were produced from crystalline alanine (standard) and from alanine separated from the other amino acids in an amino acid analyser, respectively.

Separation of protein amino acids

The crude protein content of the rumen liquor was determined by a *Kjel-Foss* 16,200 quick nitrogen analyser, then 200–500 mg material (equal to about 10–20 mg protein) depending on the crude protein content was hydrolysed with 6 mole hydrochloric acid over 24 hours. When the hydrolysis finished, the hydrochloric acid was removed by lyophilization from the sample. Separation of the protein amino acids and determination were carried out with LKB 4101 type amino acid analyser and LKB fraction collector attached to it. The test-tubes corresponding to the respective amino acid were evaporated dry by lyophilization. Then the diastereomer dipeptides were produced from the amino acids either from each separately or from several amino acids in a mixture, naturally with special attention paid to the alanine.

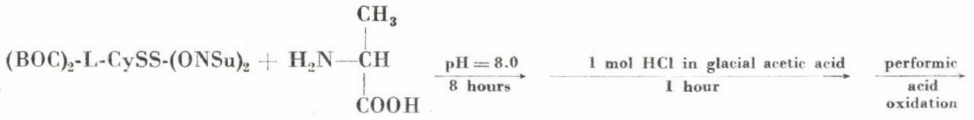
Synthesis of diastereomer dipeptides

The synthetic alanine or the residue separated in amino acid analyser and dried by lyophilization was so dissolved in water as to obtain a solution of about 1–10% concentration. The pH of the solution was adjusted to 8 with one or two crystals of sodium hydrogen-carbonate added to it, then the protected active ester of Ala or CySS, dissolved in dioxan-water 1 : 1 was added in 2 to 2.5 times excess. The reaction mixture was placed in a shaker at room temperature for 2 hours in the case of Ala and for 8 hours with CySS, then dried by lyophilization. After concentration the BOC protective group was — in both cases — split with 1 mole HCl in glacial acetic acid solution (reaction time: 1 hour), then lyophilized again. After the lyophilization the alanyl dipeptides were dissolved in citrate buffer of pH = 2.2, then after proper dilution this solution was introduced onto the ion exchange column of the amino acid analyser to separate the diastereomer dipeptides. This can all be summarized by the following reaction equations:

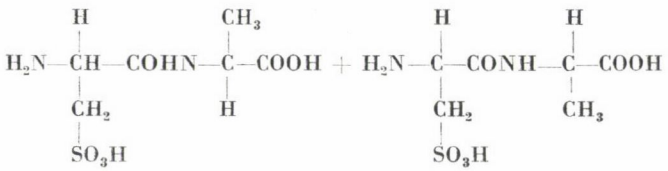


The cystinyl peptide was oxidized with performic acid after Hirs (1956). After the breakup of the disulphide bridge two dipeptides containing cysteic acid were obtained, which

after the removal of the performic acid was dissolved in a citrate buffer of pH = 2.2 and introduced onto the ion exchange column of the amino acid analyser. The concentrations were so adjusted by dilution that those of the dipeptides produced fell within the 50–100 nanomole domain. The reaction equations are:



DL alanine



DL-2-sulfonic acid-alanyl-alanine LL-2-sulfonic acid-alanyl-alanine

Separation of diastereomer alanyl-alanine, and/or 2-sulfonic acid alanyl-alenine dipeptides

The diastereomer dipeptides produced through reactions discussed in the former section were separated and determined by means of an LKB 4101 automatic amino acid analyser. The conditions of separation in the case of the alanyl-alanine dipeptides agreed with those described for the diaminopimelic acid with the difference that, since the dipeptide eluated with the B buffer, the use of the C buffer was unnecessary. In the case of 2-sulfonic acid--alanyl-alanine dipeptides, the temperature of the ion exchange column was reduced to 40 °C and the pH of the buffer to 2.9 in order to achieve an optimum separation.

Results

Diaminopimelic acid determination

According to the well-known relevant literature (Edols, 1985; Pongor and Baintner, 1980) and from handbooks on amino acid analysers at our disposal, DAPA appears in the chromatogram between valine and isoleucine, before or after methionine, or occasionally merged into methionine in the course of separation by ion exchange column chromatography. With the buffer composition suggested by Edols (1985) DAPA appears between methionine and isoleucine in the chromatogram, separation and evaluation are considered optimum until the concentration of DAPA is close to those of the other amino acids. When DAPA has to be determined from rumen liquor or intestinal content, instead of using a standard, the situation is essentially different. In some cases DAPA cannot be evaluated because of methionine and isoleucine present in high concentrations. With the above taken into consideration the sample was oxidized before the hydrochloric acid hydrolysis with performic acid produced according to the method of Hirs (1956), whereby cystine transformed into

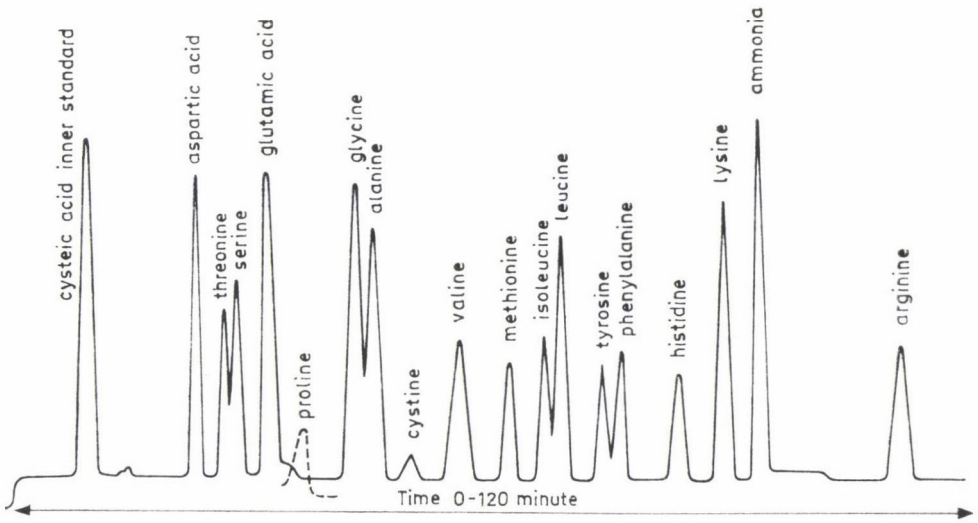


Fig. 1. Chromatogram for fishmeal

cysteic acid and methionine into methionine sulphone. In this way the place between valine and isoleucine was made free for DAPA, and with a change in the composition of buffers DAPA could be well evaluated in the chromatogram beside the amino acids, even at very low concentrations.

The amino acid chromatograms annexed provide a clear illustration of the points described above. Figure 1 stands for fishmeal, Figure 2 for fishmeal underwent performic acid oxidation, Figure 3 for DAPA, Figure 4 for DAPA after performic acid oxidation, Figure 5 for fishmeal + DAPA after oxidation. The chromatograms clearly show that the performic acid oxidation has no

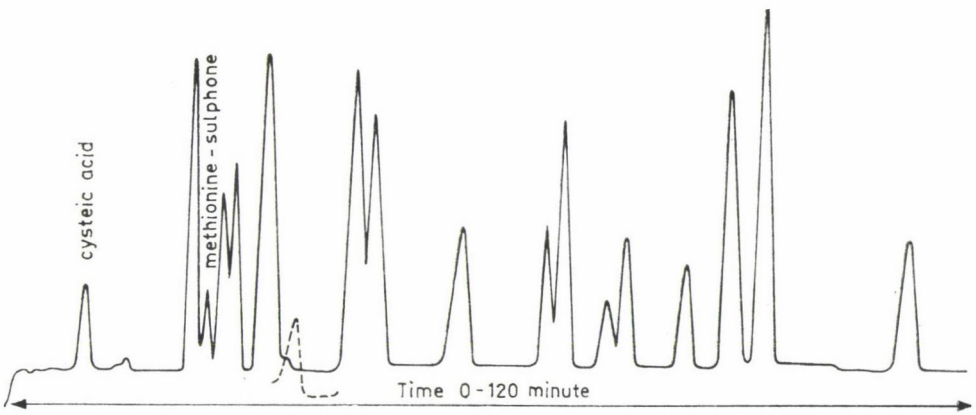


Fig. 2. Chromatogram for fishmeal underwent performic acid oxydation

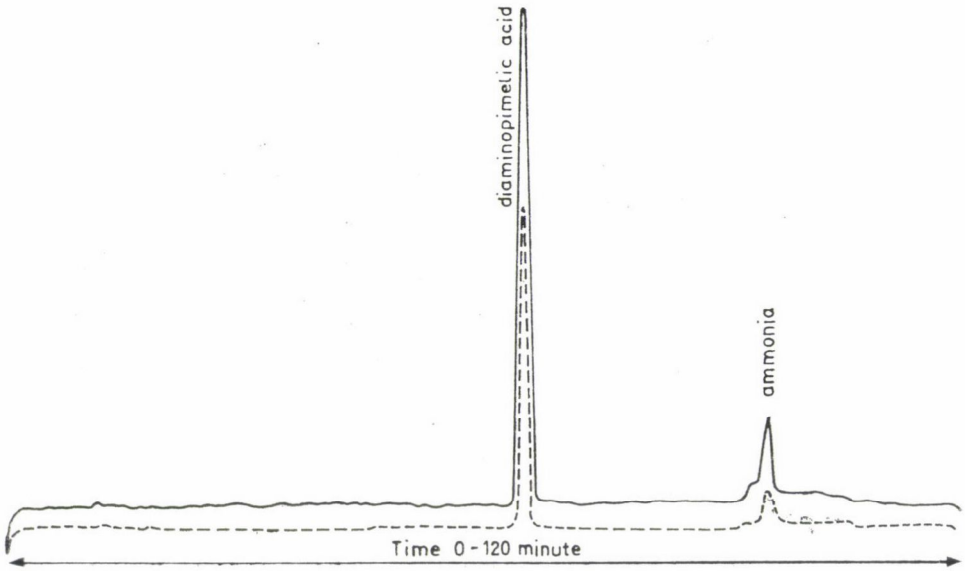


Fig. 3. Chromatogram for DAPA

practical effect on DAPA (though in the chromatogram of the oxidized sample the quantity of ammonia is slightly larger), and among the amino acids it is only the tyrosine that can be expected to decompose.

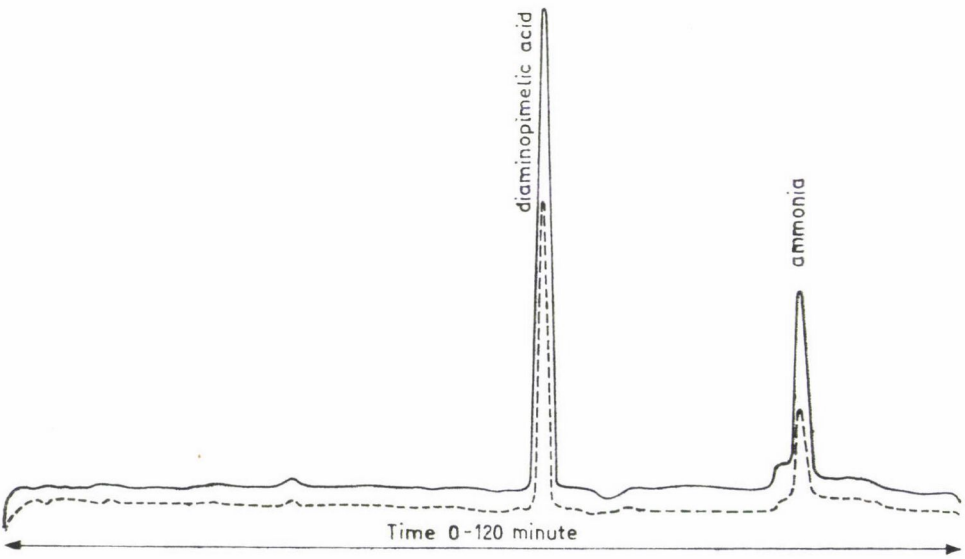


Fig. 4. Chromatogram for DAPA after performic acid oxydation

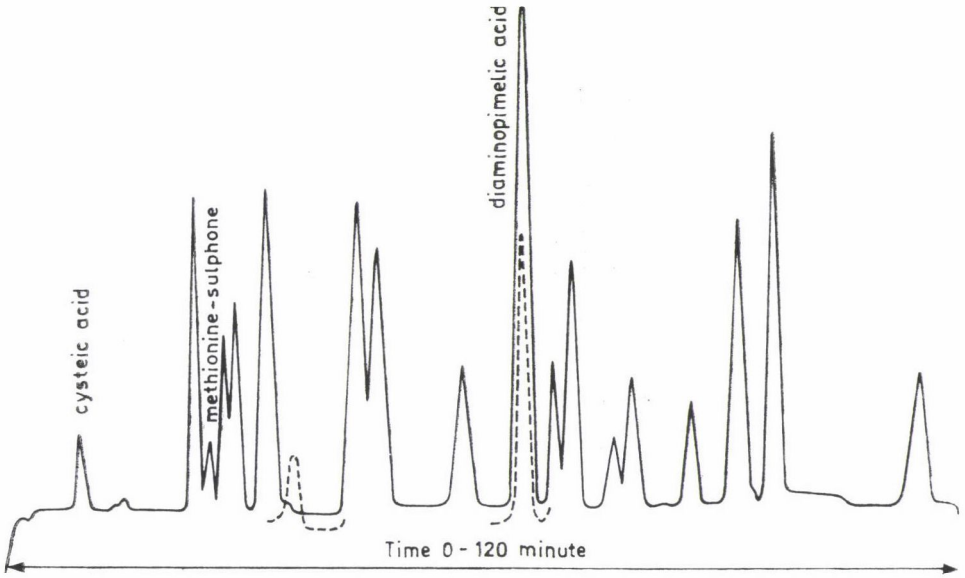


Fig. 5. Chromatogram for fishmeal + DAPA after oxydation

D-alanine determination

The separation of diastereomer alanyl-alanine- and 2-sulfonic acid-alanyl-alanine dipeptides is shown in Figures 6. and 7. In the former chromatogram, 4 distinct peaks are seen which represent: alanine used as active ester and as initial material (to be determined); L-Ala-L-Ala and L-Ala-D-Ala

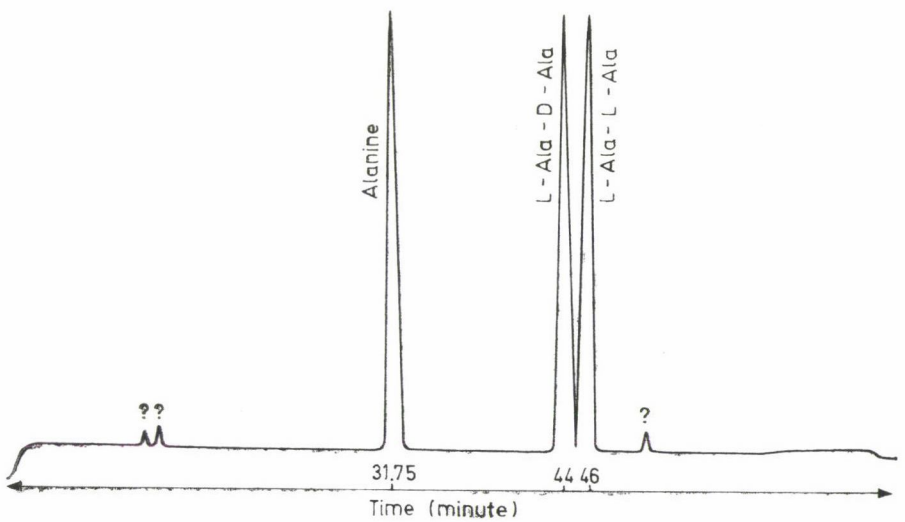


Fig. 6. Chromatogram: determination of D-Ala and L-Ala

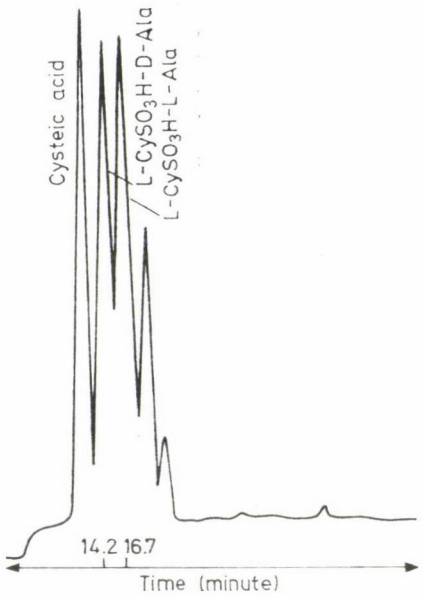


Fig. 7. Chromatogram: separation of diastereomer alanyl-alanine and 2-sulphonic acid--alanyl-alanine dipeptides

dipeptide, and at the end of the chromatogram ammonia. Under the given conditions of chromatography, the separation of the two dipeptides from one another and from the initial alanine is very good, and the evaluation is easy, since no disturbing effect makes it difficult. The only disadvantage in this procedure is that determination takes nearly an hour, and so with one amino acid analyser D-Ala content can be determined only for some 7–8 samples a day. It is here that we tried to help with the synthesis of 2-sulfonic acid-alanyl-alanine.

The incorporation of the sulfonic acid group in the dipeptide naturally quickens its elution in the course of separation by ion exchange column chromatography. In the chromatogram the cysteic acid appears in the 11th minute immediately after the front, followed in the 19th minute by a peak of a size of about 25% compared to the cysteic acid, and in the 22nd minute by one of about 5% in size, the larger of which probably represents the cysteinesulfinic acid, while the smaller peak has so far not been identified. The two mentioned peaks cause no difficulties in the case of the alanine, because the two diastereomer 2-sulfonic acid-alanyl-alanine dipeptides appear in the chromatogram on the area between the cysteic acid and the second free peak. The separation of the diastereomer dipeptides from one another and from the cysteic acid is also considered good, and with the example of the normal amino acid analysis, it can be compared to the separation of threonine-serine.

Table 1

Accuracy of D-alanine determination

Dipeptide	Theoretical value, %		Measured value, %		Number of measuring	Standard deviation		Variation coefficient	
	D	L	D	L		D	L	D	L
Alanyl-alanine	50	50	51.0	49.3	5	1.62	1.58	3.18	3.21
	25	75	25.2	75.1	5	1.05	2.05	4.17	2.73
	5	95	4.6	94.3	5	0.24	2.74	5.22	2.91
	1	99	1.10	98.8	5	0.055	2.90	5.00	2.94
2-sulfonic	50	50	49.9	51.0	5	2.98	2.63	5.97	5.16
alanyl-alanine	25	75	24.6	74.9	5	2.00	3.11	8.13	4.15
	5	95	5.1	95.2	5	0.54	4.62	10.59	4.85
	1	99	1.02	98.4	5	0.085	5.03	8.33	5.11

After elaborating the method we determined the D- and L-Ala composition for the various synthetic amino acid mixtures we produced and the results are shown in Table 1. As seen from the data in the table, with an increasing concentration the value of the variation coefficient becomes lower; that is, in a higher range of concentration the accuracy of determination increases. Despite this, in the case of the alanyl-alanine dipeptides, the value of the variation coefficient does not even reach 10 with the lowest concentrations. The method is thus reliable, and its reproducibility satisfactory. In the case of the 2-sulfonic acid-alanyl-alanine dipeptides the scatter is much wider, possibly because either the production of the dipeptide contains an additional step of performic acid oxidation, or else the separation of the diastereomer dipeptides of very short retention time is not so perfect as in the former case. The value of the variation coefficient with the 2-sulfonic acid-alanyl-alanine dipeptides exceeds 10 in a single case, and even then but slightly, so this method is also reliable and similarly well reproducible.

DAPA and D-Ala content of rumen liquor and various feeds

After the elaboration of DAPA and D-Ala analytics rumen liquor from Holstein-Friesian cows, basic materials of feed consumed by the animals (maize silage, meadow hay, lucerne hay) as well as a milking concentrate were analysed for DAPA and D-Ala. The analysis of feed components was intended to discover whether they contained considerable quantities of these two compounds, and whether in the course of preparation (acid hydrolysis at high temperature) racemization took place, and if so, what proportion of D-Ala obtained from the rumen liquor could be attributed to a possible racemization in the course of processing. The results of these analyses are contained in Tables 2 and 3.

Table 2

DAPA content of rumen liquor and various feedstuffs

Sample	Total nitrogen	DAPA	DAPA-N / Total N ×100
	g/100 g dry matter		
Maize silage	1.21	0.008	0.097
Meadow hay	1.93	0.0005	0.0074
Lucerne hay	2.88	0.0006	0.0031
Milking concentr.	2.50	0.0005	0.0029
Rumen liquor 1.	4.71	0.200	0.625
2.	4.83	0.204	0.621
3.	4.74	0.196	0.608
4.	4.79	0.194	0.596
5.	4.82	0.198	0.604

As shown by the data of Table 2 meadow hay, lucerne hay and milking concentrate only contain traces of DAPA, while the DAPA content of maize silage is some 12–15-times higher than that in the former feedstuffs, which may be explained by the microbial activity taking place in the maize silage. The DAPA contents of rumen liquors taken at different times from cows given the same feed were practically identical; 0.59–0.63% of the total nitrogen content in the rumen liquor originated from DAPA.

As seen from the data of Table 3, the D-alanine content of meadow hay, lucerne hay and milking concentrate is uniformly about 0.005%, while the corresponding value for maize silage is 0.019%, possibly due again to the microbial activity. The minimum D-Ala content in the three other feed com-

Table 3

D-Ala content of rumen liquor and various feedstuffs

Sample	Total nitrogen	D-Ala	D-Ala-N / Total N ×100
Maize silage	1.21	0.019	0.247
Meadow hay	1.93	0.006	0.049
Lucerne hay	2.88	0.005	0.027
Milking concentr.	2.50	0.005	0.031
Rumen liquor 1.	4.71	0.621	2.07
2.	4.83	0.643	2.09
3.	4.74	0.640	2.12
4.	4.79	0.639	2.09
5.	4.82	0.640	2.08

ponents is probably caused by the protein hydrolysis and by the racemization occurring in the course of processing and determination. The D-Ala content of the rumen liquor ranges between 0.621% and 0.643%, and is practically the same in samples taken at different points of time. The nitrogen content of D-Ala was 2.1% of the total nitrogen in the rumen liquor.

The analyses completed prove that under uniform feeding conditions the nitrogen content of DAPA and D-Ala form an unchanging proportion of the total nitrogen content of the rumen liquor. The precision of the method of analysis we used to determine the DAPA and D-Ala is satisfactory; in both cases equal or nearly equal to the accuracy of the normal amino acid analysis. We see no difference in accuracy and reproducibility between the two methods.

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 175–180 (1991)

CHEMICAL AND BIOLOGICAL COMPARISON OF PEA (*PISUM SATIVUM*) VARIETIES FOR SEED CROP

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(Received: 27th February 1989; accepted: 31st July 1989)

Chemical composition and protein coversion parameters were determined for 11 Hungarian and foreign bred seed pea varieties. The analyses found a 7.3–6.0 g/100 g protein lysine content, and N-metabolism tests with rats some 76% biological value and 86% true digestibility for the IP 8, IP 3, Tyrkys and Melinda varieties. The results assist the plant growers and -breeders in the selection and qualification of varieties, and call attention to the advantages of using peas as protein feed in forage mixtures.

Keywords: biological value of protein, N-metabolism, seed pea varieties

Introduction

While cereals rich in energy are available from domestic production for feeding monogastric animals, it is a problem where to find protein carriers. The imported extracted soybean does not in many cases cover the protein demand of livestock farming. For this very reason the production of protein fodders beside soybean grown in Hungary should be encouraged. For example, out of the legumes, the seed crop of pea, horse-bean and lupine can be taken into account. Their importance lies in their protein content generally being twice as much as that of the cereals. In addition, unlike the high oil content soybean, sunflower and rape seeds, they can be immediately used in fodder mixtures without further processing.

Among the fodder legumes, pea has the widest choice of variety. The joint production area of table- and fodder peas expanded from about 50,000 ha in 1981 to 130,000 ha by 1988, according to the information of the Seed Production and -Marketing Enterprise. Out of the objectives of crop growing, production for feeding purposes is considered the simplest, as conditions other than purity and nutrient content need not be considered (Bódis, 1983). For this reason, among others, one may take peas as a source of protein in fodder mixtures into consideration. Peas have a favourable effect in fodder mixtures for sows, piglets and young pigs alike, and they can be substituted generally for 35–40% of the soybean protein without reducing the performance of the animals (Fuchs et al., 1983; Davies, 1984; Lund and Hakansson, 1986).

Zivkovic et al. (1987) report replacing 50% of the soybean by peas in a fodder mixture for piglets, and completing it with tryptophan, with the result of a 39% body weight increase. Owing to the rising prices of import fodders, Ceasar (1987) in the German Federal Republic also tried to find fodder crops rich in protein suitable to replace them. In his opinion, peas due to their protein- and lysine contents must by all means be taken into account beside fishmeal and extracted soybean.

Consequently, the production of peas in Hungary should be increased with the possibly highest yielding and high nutrient content varieties. In the case of fodder peas, the choice of variety has to be determined by the nutrient content and the biological value of protein.

Our study was intended to compare the registered and commercially produced pea varieties placed at our disposal by the Seed Producing and Marketing Enterprise for their chemical compositions and biological values. The results obtained may help in selecting varieties best suiting the purpose of feeding.

Materials and methods

We determined the nutrient content (dry matter, crude protein, crude fat, crude fibre, ash) in seeds of 11 Hungarian and foreign pea varieties according to the MSz 6830 standard. The amino acid composition of the protein was determined using an Aminochrom-II. type amino acid analyser.

On the basis of N-metabolism tests with rats (Szelényi, 1969) we obtained the biological value, digestibility, net- and productive utilization of the pea proteins.

Results

Chemical analyses

In Table 1 the percentage nutrient contents of the 11 pea varieties examined are related to the same (86%) dry matter content. Most remarkable is the relatively low crude protein content in the peas examined. It is only in the varieties Trapper and Solara that the crude protein contents (22.8% and 21.5%, respectively) come close to the 23% average of the Hungarian Standard. Particularly low crude protein contents were found in the varieties IP 8, Smaragd and Tyrkys (16.5–17.5%).

The crude fat contents of the peas are negligible (0.9–1.6%), though the varieties show considerable differences.

Another advantage of the pea is its low crude fibre content. The values found by us for this component were between 3.0% and 5.8%.

The ash content ranged between 2.6 and 3.0%, the N-free extracted matter between 55.8% and 60.8%; both are close to the Hungarian averages.

Since the pea belongs to the protein feeds, its amino acid composition of protein is particularly important. Table 2 contains the amino acid composi-

Table 1

Percentage nutrient content of seed in pea varieties
(related to 86% dry matter)

Pea variety			Crude protein	Crude fat	Crude fibre	Ash	N-free extr. m.
Name	Origin	Ripening time	content				
Melinda	Hungarian	medium	19.9	1.1	4.7	2.6	57.7
IP 8.	Hungarian	early	16.5	1.4	5.8	3.0	59.3
IP 3.	Hungarian	early	19.1	1.6	5.7	2.7	56.9
Allround	Dutch	medium	18.5	1.3	4.0	2.8	59.4
Auralia	GDR	medium	19.7	1.1	3.0	2.6	59.6
Solara	Dutch	medium	21.5	1.0	3.9	3.0	56.6
Imposant	Dutch	medium	19.1	1.5	4.1	2.6	58.7
Botond	Hungarian	medium	19.9	1.0	3.3	2.9	58.9
Tyrkys	Czechoslov.	medium	17.5	1.2	4.9	2.7	59.7
Smaragd	Czechoslov.	medium	16.9	1.1	4.6	2.6	60.8
Trapper	Canadian	medium	22.8	0.9	3.7	2.8	55.8

Table 2

Amino acid composition of pea varieties g/100 g protein

Amino acids	Variety										
	Melinda	IP 8.	IP 3.	All-round	Auralia	Solara	Impo-sant	Botond	Tyrkys	Sma-ragd	Trapper
Aspartic acid	7.9	12.5	12.2	11.4	11.6	10.8	12.4	13.0	12.0	11.4	12.2
Threonine	3.7	4.0	3.4	3.3	3.3	3.0	3.5	3.4	3.4	3.8	3.7
Serine	3.9	3.7	3.8	4.7	3.4	3.9	3.3	3.6	4.1	3.6	4.2
Glutamic acid	17.1	13.3	14.6	15.2	15.6	15.7	15.9	15.8	16.9	16.6	16.0
Proline	11.1	12.6	14.1	14.7	15.0	12.6	15.7	14.1	14.1	12.5	14.1
Glycine	3.7	4.3	4.1	3.9	3.7	3.8	4.0	3.4	3.4	4.2	4.6
Alanine	3.7	4.3	3.9	4.4	3.5	5.3	3.5	3.4	3.7	4.9	5.1
Cystine	1.0	1.2	1.0	1.1	1.4	1.5	1.6	1.0	1.0	1.1	1.7
Valine	4.6	5.1	4.8	5.2	4.5	6.3	4.4	4.5	4.2	4.6	6.3
Methionine	1.1	1.3	1.2	1.5	1.5	1.4	2.1	1.3	1.2	1.2	1.3
Isoleucine	3.7	3.6	3.9	3.4	3.8	3.9	3.9	3.5	2.8	3.1	3.8
Leucine	8.2	7.8	6.9	8.5	8.7	8.2	8.7	8.9	8.1	8.0	8.3
Tyrosine	3.2	4.0	3.2	3.7	3.5	3.2	3.5	3.1	3.5	3.6	2.9
Phenylalanine	6.5	7.0	5.5	5.5	5.4	5.1	5.1	5.7	5.3	4.5	5.1
Lysine	6.4	7.3	6.8	5.8	6.2	5.3	5.9	4.9	6.6	6.0	5.8
Histidine	2.9	3.6	2.6	3.3	2.8	2.5	2.8	2.4	2.8	2.8	2.3
Arginine	6.5	5.9	8.1	6.5	6.7	7.5	6.9	6.1	7.2	8.9	7.3

tions of the 11 pea varieties examined. For pigs lysine is the most important essential amino acid, which can be found in the pea protein in considerable amounts. The highest lysine content was determined for the varieties IP 8 (7.3 g/100 g protein) as well as for IP 3, Tyrkys, Melinda, Auralia and Smaragd (6.8–6.0 g/100 g protein), while the least lysine was contained in the variety Botond (4.9 g/100 g protein). Outstandingly high methionine content (2.1 g/100 g protein) was found in the variety Imposant, while in the other varieties the methionine content ranged from 1.1 to 1.5 g/100 g protein. The cystine content was outstanding in the varieties Trapper and Imposant (1.7 and 1.6 g/100 g protein, respectively), while in the other peas this amino acid ranged between 1.0 and 1.5 g/100 g protein.

Biological examinations

The protein conversion parameters obtained in N-metabolism test with rats are shown in Table 3. The best biological value appears in the variety Imposant (79%). Only slightly lower were the values obtained for IP 8, Tyrkys and Smaragd (76.6–76.8%). The poorest biological value applies to the proteins of the varieties Trapper and Solara (65.8–68.1%).

The true digestibility of protein gave the lowest values (83.4–85.9%) for the varieties Botond, Imposant, Tyrkys, IP 3, Melinda and IP 8, and the highest values (87.2–88.4%) for Smaragd, Allround and Trapper.

Table 3

Major protein utilization parameters for pea varieties
%

Pea variety	Protein			
	Biological value	True digestibility	net utilization	productive utilization
Melinde	74.6	85.2	63.6	40.7
IP 8.	76.6	85.9	65.9	40.5
IP 3.	72.0	84.2	60.6	38.4
Allround	75.2	87.6	65.9	43.4
Auralia	66.8	86.7	57.8	35.0
Solara	68.1	86.3	58.8	36.0
Imposant	79.0	84.4	66.7	43.7
Botond	71.6	83.4	59.6	35.6
Tyrkys	76.7	84.8	65.0	41.3
Smaragd	76.8	87.2	66.8	45.2
Trapper	65.8	88.4	58.2	34.8

The net utilization of protein showed a similar tendency as the biological value; that is, Imposant, Smaragd, Tyrkys, IP 8 and Allround were the best, and Auralia, Solara and Trapper the poorest varieties in this respect.

The productive utilization of protein did not quite follow the tendency of the biological value; namely, the best productive conversion was shown by the varieties Smaragd, Imposant and Auralia, in the given order.

Conclusions

Both the chemical analyses and the biological examinations provide reasons for a wider introduction of pea seed as a protein feed. For crop producers the primary aim has so far been to attain the largest possible yields. However, the aspect of breeding must by all means be widened — insofar as feeding is the aim of production — by taking into consideration the nutrient content and its convertability. The protein contents and amino acid compositions given on the basis of our chemical analyses, as well as the protein conversion parameters of the metabolism tests with rats, assist in qualifying the varieties and choosing the right ones.

After these preliminary remarks we suggest first of all to cultivate the varieties IP 8, IP 3, Tyrkys and Melinda for the purpose of feeding pigs, owing to their high lysine contents and biological values. The biological value of protein in the varieties IP 8, Tyrkys and Melinda is significantly better ($P < 0.01$) than in the variety Trapper, certainly due to the considerable proportion of lysine out of the essential amino acids. Since among pig feeds lysine has the greatest importance, the cultivation of the three mentioned varieties and their use in fodder mixtures is — on the basis of our results — recommended. The Dutch variety Imposant — though qualified best from a feeding standpoint — is not mentioned among those suggested by us, because according to the information of the Seed Producing and Marketing Enterprise it is planned to be downgraded; its seed breaks when thrashed, and there are problems with the production of seed.

Our examination results emphasize the aspects of feeding, but it is quite natural that the aspects of crop production and utilization must be jointly taken into consideration when choosing a pea variety and introducing it in commercial production. Those pea varieties found to be the best from the point of view of both crop production and feeding should be further examined in metabolism experiments with pigs. In that way protein conversion from the best varieties — i.e. protein incorporation and muscle formation, which serve for meat production, could be proved by the results of the suggested experiments.

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 181–191 (1991)

Animal Production and Genetics

EFFECT OF SALINOMYCIN AND HIGH RATE CONCENTRATE DIET INTAKE ON RUMINAL FERMENTATION IN LAMBS

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(Received: 13th April 1989; accepted: 26th September 1989)

The author compared the effects of salinomycin and high rate concentrate diet intake on some physiological parameters of growing lambs. On the basis of the results the author established that the salinomycin had a considerable influence on the metabolism of both carbohydrate and nitrogen in the rumen, since the amount of propionic acid increased while the molar ratio of butyric acid and the ammonia-N-and urea content of the rumen fluid decreased. Out of the parameters of blood examined, the glucose concentration of the blood increased, while the urea content of the plasma was significantly lower with the application of salinomycin. The experimental groups of animals did not show essential differences in the examined parameters of rumen and blood with a higher rate of concentrate diet intake.

Keywords: concentrate diet intake, ammonia-N, lambs, ruminal fermentation, volatile fatty acids, salinomycin, blood glucose

Introduction

Recent results of investigations suggest that there are wide possibilities of increasing the feed conversion of ruminants by influencing or controlling the ruminal digestion.

From feedstuffs rich in cellulose first of all acetic acid, while from those abounding in sugar and starch much propionic acid, is formed. Therefore, in beef- and lamb fattening (meat production) with feedstuffs rich in starch (e.g. concentrate diet) an optimum medium can be ensured for bacteria producing propionic acid. The fermentation of propionic acid in the rumen can be promoted by providing various compounds. Such are, among others, the antibiotic ionophores, the use of which in animal production is of increasing importance owing to their favourable effect on body mass gain and feed conversion. This is the case with salinomycin, the compound tested in our experiments.

Salinomycin (Salocin 120^{R}, Hoechst Ag, GFR) given to ruminants as a growth promotant has been tested by a number of authors (Piva et al., 1981;

Bedő et al., 1985; Merchen and Berger, 1985; Kobayashi et al., 1986; Droumev et al., 1988).

Despite the large number of works published so far, the effect of salinomycin on the ruminal digestion of ruminants — with special regard to changes observed in the N-metabolism of the rumen — has not been made perfectly clear. According to some authors the ammonia content of the rumen increased with the application of salinomycin (Piva et al., 1981), while others did not find any significant change in the ammonia- and urea concentration of the rumen (McClure et al., 1980). Webb et al. (1980) observed a decrease in the ammonia-N content of the rumen on feeding salinomycin. The conflicting reports suggest a correlation between salinomycin as an active substance and the feed that forms the substrate, that is, it makes a difference what kind of feed is given when the effect of salinomycin is studied. This is supported by the results of our earlier experiment with growth promotants (Fébel et al., 1988).

The experiment here described was intended to study the way salinomycin influenced the ruminal fermentation in lambs, and through it, some physiological parameters of the blood in case the animals in the course of fattening exclusively consumed a concentrate diet ("monodiet" feeding) in large amounts.

Materials and methods

The experiment was carried out with 16 rumen fistulated young Hungarian combing merino rams. The average body weight of the animals at the beginning of the experiment, at 12 weeks of age, was 22.7 $\pm$ 1.4 kg.

The composition and nutrient content of the diet during the experiment are shown in Table 1.

The chemical analysis of the diet was made according to the prescriptions of the current Hungarian standard (MSZ 6830), and its net energy value (NEm and NEg) was calculated on the basis of the TDN-value of the components. The feed was given daily in granulated form in two parts: at 8.30 a.m. and at 4 p.m. The refusal was measured back every day.

In the experiment four groups (*A*, *B*, *C* and *D*) were formed, each with 4 animals.

The different rates of concentrate intake by the groups were so achieved that the net energy in the ration given to groups *A* and *C* corresponded daily to 100 g, and in that fed to groups *B* and *D* to 200 g body weight gain.

On the basis of weekly weighing results the feed rations were so increased as to ensure the prescribed net energy intake for the lambs.

The salinomycin treatment (24 mg salinomycin/kg feed) was used in groups *C* and *D*, so groups *A* and *B* were regarded as controls in this respect.

The daily dry matter-, crude protein- and energy intake by the lambs are shown in Table 2. The data show that the daily body mass gains of groups *A* and *C* were 100 g, while in group *B* 178 and in group *D* 183 g were in terms of the energy intake of animals.

The experiment lasted 4 weeks. In the 2-week experimental period following a 2-week period of prefeeding, rumen fluid- and blood samples were taken on the 5th, 9th and 13th day before feeding, and 2 and 5 hours after feeding. In the rumen fluid samples, the pH-value and the ammonia-, urea- and lactic acid concentrations, and in the rumen fluid taken in the 5th hour after feeding the volatile fatty acid concentration too, were determined. The blood samples were taken at the above mentioned sampling time (before feeding and 2 and 5 hours after feeding) and the samples were assayed for ammonia-, urea- and glucose contents.

The pH of the rumen fluid was determined electrometrically with a Radiometer PHM-27 type pH meter (Copenhagen); its ammonia- and urea concentrations by Berthelot's method

Table 1

Composition and nutrient content of the diet fed in the experiment

Components	Composition (%)	Nutrient content
Meadow hay	5.0	
Maize	42.5	
Wheat	25.0	
Alfalfa meal, 2nd rate	7.0	
Extr. sunflower	19.5	
MH-03 premix	0.5	
Salt (NaCl)	0.5	
Total	100.0	
Dry matter, g		875
Crude protein, g/kg DM		182
Crude fibre, g/kg DM		100
Crude fat, g/kg DM		35
NE_m, MJ/kg DM		7.08
NE_g, MJ/kg DM		4.55

(Klinisches Labor. Merck, 1974), while its lactic acid concentration was measured by the photometric micromethod of Velősy (1979). The volatile fatty acids were analysed, after precipitation with metaphosphoric acid, by a Chrom-41 gas chromatograph (Supelco Bulletin 749B, 1975). The stationary phase was GP 10% SP-1200/1% H_3PO_4 supported on a Chromosorb^R WAW 80/100 mesh.

The ammonia content of blood and the urea content of plasma were assayed by the above described methods. The concentration of blood glucose was determined using o-toluidine as a reagent (Szilágyi, 1971).

The comparison and evaluation of the results were carried out by a variance analysis and a t-test (Sváb, 1981).

Table 2

Trend of nutrient intake during the experiment
(n = 4/each)

	Groups			
	A	B	C	D
Dry matter, g/day	801	1024	812	1082
Crude protein, g/day	145	186	147	196
NEm (requirement), MJ/day	3.45	3.44	3.48	3.53
NEg (available), MJ/day	1.43	2.45	1.45	2.65
Body weight gain per ration, g/day	100	178	100	183

Results

The changes in the ruminal fermentation processes are summarized in Tables 3 and 4.

As seen from Table 3, before and 2 h after feeding, the pH values of the rumen fluid were nearly the same in all groups. A significant difference was only found between groups *C* and *D* at the last sampling time.

The ammonia-N- and urea contents of the rumen fluid were considerably influenced by the salinomycin treatment; in groups *C* and *D* the concentrations of both parameters were significantly lower at each sampling time than in groups *A* and *B*.

No difference in the ruminal concentration of lactic acid was shown by the groups at the 1st and 2nd time of sampling. The highest lactic acid content 5 h after feeding was measured in group *D* (1.11 mmol/l).

Changes observed in the volatile fatty acid composition of the rumen fluid are shown in Table 4.

The total volatile fatty acid concentration gave nearly identical values in all experimental groups. Within this the greatest changes were found in the concentrations and molar proportions of the acetic acid and propionic acid, and in the acetic acid: propionic acid ratio.

The salinomycin treatment decreased the molar proportion of the acetic acid and increased that of the propionic acid in groups *C* and *D*, consequently, the ratio of acetic acid to propionic acid decreased in these groups.

The concentration of i-butyric acid in group *C* was significantly lower than in groups *A* and *D*. As for the molar proportion of i-butyric acid, the groups showed no difference.

The concentration and molar proportion of the butyric acid were significantly lower in groups *C* and *D*.

The highest i-valeric acid- and valeric acid contents of the rumen fluid were found in group *B*. In animals fed with salinomycin, the concentration and molar ratio of valeric acid significantly decreased.

Changes in the glucose-, ammonia- and urea concentrations of the blood samples are shown in Table 5.

The blood glucose level in the groups feeding on salinomycin (*C* and *D*) was significantly higher 2 and 5 h after feeding. The blood ammonia content before feeding was practically the same in all experimental groups. The highest ammonia concentrations were measured in group *B* 2 h after feeding while in group *D* at the last sampling time, but these values did not exceed the upper physiological limit. The urea concentrations of the plasma were significantly lower in groups *C*- and *D*-, than those of the *A*- and *B*- at each sampling time.

Table 3

Changes in the pH value and ammonia-N-, urea- and lactic acid concentration of the rumen fluid at the 1st, 2nd and 3rd time of sampling
($\bar{x} \pm$ LSD)

Group	pH			Ammonia-N mmol/l			Urea mmol/l			Lactic acid mmol/l		
	1.	2.	3.	1.	2.	3.	1.	2.	3.	1.	2.	3.
A	6.49 ±0.14	5.04 ±0.16	5.53 ±0.21	4.44 ±1.22	6.82 ±1.91	5.89 ±0.79	6.56 ±1.19	6.53 ±1.47	6.48 ±0.99	1.00 ±0.12	1.01 ±0.20	0.96 ±0.09
B	6.33 ±0.19	5.21 ±0.19	5.67 ±0.23	4.72 ±1.49	6.68 ±2.34	5.55 ±2.10	6.26 ±1.18	5.83 ±2.20	5.70 ±1.72	0.99 ±0.22	0.86 ±0.19	0.89 ±0.15
C	6.51 ±0.31	5.02 ±0.19	5.49 ±0.21	3.76 ±0.71	3.57 ±0.71	3.43 ±0.74	3.39 ±0.34	2.96 ±0.75	2.59 ±0.48	0.95 ±0.18	1.02 ±0.20	1.03 ±0.20
D	6.67 ±0.33	5.10 ±0.12	5.73 ±0.27	3.62 ±0.74	3.41 ±0.98	3.72 ±0.79	2.87 ±0.96	2.50 ±0.50	2.47 ±0.72	0.94 ±0.26	0.98 ±0.21	1.11 ±0.26
Significance level of group differences	NS	NS	C—D $P < 0.01$	A—C $P<0.01$ B—D $P<0.01$	A—C $P<0.001$ B—D $P<0.001$	A—C $P<0.001$ B—D $P<0.001$	A—C $P<0.001$ B—D $P<0.001$	A—C $P<0.001$ B—D $P<0.001$	A—C $P<0.001$ B—D $P<0.001$	NS	NS	B—D $P<0.01$

Note: 1st time of sampling — before feeding
2nd time of sampling — 2 hours after feeding
3rd time of sampling — 5 hours after feeding

Table 4

Changes in the volatile fatty acid concentrations of the rumen fluid and in the molar proportions of the volatile fatty acids 5 hours after feeding
($\bar{x} \pm$ LSD)

Parameters examined	Groups				Comparison of the groups by t-test	
	A	B	C	D		
Volatile fatty acid, mmol/l						
Total volatile fatty acid	107.72±19.19	108.41±11.69	100.44±20.80	108.78±12.50	NS	
Acetic acid	43.96±8.84	46.01±5.59	34.90±9.40	36.31±5.60	AC**	BD***
Propionic acid	30.62±7.50	30.63±6.63	42.28±8.76	47.42±5.38	AC**	BD***
i-butyric acid	2.52±0.87	2.40±0.54	1.91±0.38	2.50±0.47	AC*	CD**
Butyric acid	25.24±5.32	22.78±3.64	17.27±5.20	17.92±7.38	AC***	BD**
i-valeric acid	1.76±0.40	2.12±0.43	1.35±0.28	1.51±0.22	AB*	BD**
Valeric acid	3.62±1.03	4.46±1.09	2.72±0.66	3.12±0.83	AC*	BD*
Volatile fatty acid, mol %						
Acetic acid	40.77±3.94	42.41±2.86	34.47±3.96	33.53±4.78	AC**	BD***
Propionic acid	28.33±3.99	28.21±4.29	42.28±4.06	43.64±2.34	AC**	BD***
i-butyric acid	2.32±0.63	2.21±0.64	1.91±0.16	2.32±0.46	NS	
Butyric acid	23.54±3.18	21.05±2.96	17.18±4.23	16.25±5.47	AC**	BD**
i-valeric acid	1.65±0.33	1.92±0.44	1.39±0.33	1.39±0.18	BD*	
Valeric acid	3.38±0.82	4.15±1.04	2.77±0.75	2.86 0.65	AC*	BD*
$C_2 : C_3$	1.48±0.34	1.50±0.30	0.82±0.14	0.11±0.12	AC***	BD***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Conclusions

The different rates of concentrate intake — similar to previous observations (Putnam et al., 1966; Adams and Kartchner, 1984) — did not cause any significant difference in the pH value of the rumen fluid. The salinomycin treatment — in accordance with earlier reported data (Webb et al., 1980; Nakashima et al., 1982; Merchen and Berger, 1985; Kobayashi et al., 1986) — also failed to alter the pH value. 2 h after feeding, when fermentation started, the pH value first fell (to about 5.0), then began to rise in all groups. Then, 5 h after feeding the pH value of the rumen fluid was 5.53 and 5.49 in groups *A* and *C*, and 5.67 and 5.73 in the *B*- and *D*- groups, respectively. Since concentrate diets contain large amounts of carbohydrates which easily decompose the pH of the rumen fluid shifted towards the acid values, but the groups did not show differences in the concentration of lactic acid.

The high level concentrate intake did not bring about any increase in the total volatile fatty acid content of the rumen fluid — unlike the experiments of Staples et al. (1984) and Firkins et al. (1986a).

Neither did the application of salinomycin cause any significant changes in the total volatile fatty acid concentration of the rumen fluid, corresponded well with those obtained by Nakashima et al. (1982) and Merchen and Berger (1985).

In the course of feeding concentrate diet, the lower pH is favourable mainly for those microorganisms which promote the propionic acid fermentation of carbohydrates, while the quantity of acetic acid bacteria decreases. This was confirmed by our results obtained in the course of examining the volatile fatty acids of the rumen fluid since, in each experimental group, the acetic acid: propionic acid ratio was much lower than the 3 : 1 ratio obtained with feeding mostly based on a mixed diet. In group *A* the ratio of acetic acid to propionic acid was 1.48, in group *B* 1.50.

Salinomycin feeding (in the *C*- and *D*-groups) further decreased the acetic acid: propionic acid ratio (to 0.82 and 0.77, respectively), which indicates—in agreement with the data obtained by Webb et al. (1980), McClure et al. (1980), Piva et al. (1981), Nakashima et al. (1982), Galbraith et al. (1983), Merchen and Berger (1985) and Droumev et al. (1988) — that the propionic acid fermentation increased with a simultaneous decrease in the acetic acid production.

Enhancing propionic fermentation in the rumen in response to salinomycin feeding is of physiological importance from two points of view. On the one hand, due to its glycogenetic nature more glucose can be produced from it in the organism. This was indicated in our experiment by the rising level of the blood glucose (in the *C*- and *D*-group), which corresponds to the observation of Droumev et al. (1988). It should be noted here, that different levels of

Table 5

Trend of the blood parameters examined at the 1st, 2nd and 3rd sampling time
($\bar{x} \pm$ LSD)

Group	Blood glucose (G) mmol/l			Blood ammonia μmol/l			Urea (PU) mmol/l		
	1.	2.	3.	1.	2.	3.	1.	2.	3.
A	3.25 ±0.25	3.21 ±0.31	3.24 ±0.19	92.81 ±10.90	82.43 ±7.78	90.40 ±11.65	3.36 ±0.57	3.24 ±0.64	2.73 ±0.48
B	3.34 ±0.23	3.41 ±0.25	3.30 ±0.12	93.82 ±10.90	92.32 ±6.94	86.45 ±11.22	3.16 ±0.50	3.22 ±0.50	2.58 ±0.51
C	3.64 ±0.15	3.64 ±0.23	3.67 ±0.22	93.99 ±12.80	90.41 ±11.77	88.74 ±15.12	2.14 ±0.31	2.24 ±0.33	2.03 ±0.23
D	3.50 ±0.23	3.68 ±0.25	3.65 ±0.13	95.95 ±12.44	91.84 ±8.65	106.01 ±14.95	2.51 ±0.28	2.50 ±0.24	2.14 ±0.15
Significance level of group differences	A—C $P < 0.001$	A—C $P < 0.001$ B—D $P < 0.001$	A—C $P < 0.001$ B—D $P < 0.001$	NS	A—B $P < 0.05$	B—D $P < 0.001$ C—D $P < 0.001$	A—C $P < 0.001$ B—D $P < 0.01$	A—C $P < 0.01$ B—D $P < 0.01$	A—C $P < 0.001$ B—D $P < 0.05$

Note: See Table 3.

concentrate intake did not result in any significant change in the blood glucose concentration.

On the other hand, the increased production of propionic acid is important because it leads to H_2 uptake in the rumen, and thereby, the energetically unfavourable methane production decreases. Webb et al. (1980) demonstrated by experiments that, in response to salinomycin application, the amount of methane in the rumen decreased.

The salinomycin treatment changed the molar proportion of the other volatile fatty acids too, with the exception of the isobutyric acid.

As seen from the data of Table 4, the percentage concentration of butyric acid was found — in agreement with a number of authors (Fontenot et al., 1980; McClure et al., 1980; Nakashima et al., 1982; Galbraith et al., 1983; Merchen and Berger, 1985) — to decrease, and the molar proportion of valeric acid was similarly lower in the groups *C*- and *D*- — as was also indicated by Galbraith et al. (1983).

The decreased molar proportion of butyric acid is favourable from an energetical point of view, since from the smaller amount of butyric acid less methane can be produced in the rumen.

As known from studies on the effect of feed intake on the N-balance of the rumen, with a higher rate of feed intake the ammonia-N concentration of the rumen fluid decreases (Staples et al., 1984; Firkins et al., 1986b), because under the influence of the increased amount of carbohydrate available with the larger ration of feed the microorganisms living in the rumen incorporate the ammonia into their own proteins much more efficiently. In our experiment — as in the experiment of Hodgson et al. (1976) — this effect could not be demonstrated, since despite the higher rate of concentrate intake (groups *B* and *D*) the ammonia-N content of the rumen fluid did not change (compared to groups *A* and *C*). The unchanged ammonia-N concentration of the rumen related to the fact that the groups did not show differences in the total volatile fatty acid content of the rumen fluid.

As a result of feeding salinomycin the ammonia-N concentration as well as the urea content of the rumen fluid were lower in the *C*- and *D*-, than in the *A*- and *B*-groups.

Webb et al. (1980), Nakashima et al. (1982), Galbraith et al. (1983) and Droumev et al. (1988) reported decreased ammonia-N concentration in the rumen after salinomycin feeding. Other authors, on the other hand, either did not observe any change in the NH_3 concentration of the rumen fluid (Kobayashi et al., 1986) or even found that the ammonia level increased (Piva et al., 1981).

In our experiment the ammonia-N content of the rumen in the groups *C*- and *D*- was lower despite the fact that in these two groups the urea utiliza-

tion increased under the influence of salinomycin, as indicated by the reduced urea concentration of the rumen fluid.

The decrease in the ammonia content of the rumen fluid is explained by the lower extent of protein- and amino acid decomposition or the increased protein synthesis. It is also possible that the two processes took place simultaneously. According to the opinion of several authors (Piva et al., 1981; Kobayashi et al,. 1986) salinomycin — just like monensin — decreases desamination in the rumen. Thus, since the amount of feed protein entering the small intestines from the rumen increases, a "protein saving" effect is produced. At the same time, Droumev et al. (1988) found when feeding salinomycin an increased amount of microbial protein in the duodenum, which suggests that the protein synthesis increased in the rumen.

Despite the fact that the ammonia-N level which we measured in the rumen fluid on feeding salinomycin agreed with the ammonia-N concentration (3.5–4 mmol/l) considered as optimum in the literature (Satter and Slyter, 1974; Veen, 1986; Polan, 1988), it is likely that owing to the high level of readily fermentable carbohydrate present in the concentrate, and the reduced desamination concurrent with the increasing protein synthesis, the amount of ammonia-N was not "too much" for the microbes, as suggested by the decreased urea concentration of the plasma in the groups *C-* and *D-*. Since the microorganisms had an increased demand for ammonia, the role of the ruminohepatic circulation increased. In the ruminohepatic circulation the urea "influx" through the rumen epithelium may be 4–8 times as much as the amount of urea returned with the saliva (Karsai, 1982).

In summary, it can be established that the salinomycin increased the propionic acid fermentation and decreased the amount of acetic acid and butyric acid, and urea and ammonia-N concentration in the rumen, irrespective of the rate of concentrate intake. Out of the blood parameters examined the glucose concentration of the blood significantly increased, while the urea concentration of the plasma decreased, in response to salinomycin feeding. Simultaneously, the groups did not show essential differences in the parameters of rumen fluid and blood as a result of a higher rate of concentrate intake.

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 193–200 (1991)

Lectures

SPICE AND MEDICINAL PLANT PRODUCTION AND PROCESSING IN HUNGARY*

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(Received: 19th March 1990; accepted: 21st May 1990)

On the basis of a long-term average of medicinal plant production and consumption, countries can be divided into two major groups (Figure 1). Accordingly, the West-European countries and the USA are countries, in which the consumption of medicinal plants far exceeds the value of their own production. Conversely, the East-European countries, including Hungary, can be characterized by a production well exeeding their own consumption, implying that these countries have become traditional suppliers of medicinal drugs on the international markets.

Natural conditions

Geographical conditions

Hungary occupies the centre of the Carpathian basin. More than half of her surface is lowland, no more than 200 meters above sea level. Hills are relatively scarce, thus there are only a few countries in the world where the proportion of agricultural and arable land is as large, with respect to the entire area (93,000 km^2) as in Hungary. (More than 85% of the land is cultivated.)

Climatic conditions

As for weather systems, Hungary lies at the conjunction of three major European climatic regions; the Atlantic, the Mediterranean and the Continental. While the characteristic features of all three climates can be found alike, the continental influence predominates; cold winter and dry, hot summer with frequent droughts The annual mean temperature varies between $+7$ — $+12$ °C and the prevailing average value of precipitation amounts to

* Part of the lecture given by author at the 4th National Herb Growing and Marketing Conference, July, 22–25, 1989, San Jose, California.

620 mm/annum The annual total insolation for the country varies between 80 and 110 Kcal/cm². (The sum of sunshine-hours amounts to an average of 2000 h.)

Vegetation

Approximately 50% of the roughly 2200 flowering species native to Hungary belong to the group of European and Eurasian floristic elements. Apart from these, the rate of both continental — eastern elements (approx. 13%) and Mediterranean — Submediterranean elements (approx 16%) is also significant. Among them, some 1000 species are known to have medicinal properties: about 300 species used for this purpose include 170–180 species of medicinal plants growing wild.

The main characteristics of production

Historical background

In Hungary, the production and usage of spice and medicinal plants have historic traditions. The earliest reports on Hungarian medicinal plants, either grown in cloister gardens or utilized in healing practices date from medieval times. Although it would be truly interesting to survey this history, due to the lack of time we must content ourselves with more contemporary developments.

The decades between the two world wars witnessed a real boom both in the collection and cultivation of medicinal plants. At this time, Hungary was very successful in agricultural production, and was even called "the granary of Europe". The extent of medicinal plant production can be characterized by the following data. While between 1920–25 an average of 850 tons of medicinal plants were exported, in 1943, the Hungarian exports amounted to some 10,100 tons (5). Within the exports:

— the wild growing medicinal plants, among them mainly Chamomile flower had a substantial share (in 1920 some 180 tons, in 1940 some 780 tons). Other important items were: Nettle leaf, Rose hips, Elderberry flower, Juniper fruit, Belladonnae folium, Malvae radix and M. folium.

— Cultivation. In the 1940s, on some 6000 hectares, approximately 20–25 spice and medicinal plant species were cultivated (5.) In this year the export of peppermint, the most important crop cultivated, was some 1.780 tons.

While the pre-war times involved, mainly the collection of wild-growing drugs and the production of certain crops characteristics of Hungary (e.g. peppermint, marjoram), the later decades have brought about the large scale production of an increasing number of species (some of them introduced into cultivation). Table 1 provides a list of the 55 species cultivated on an area of

22,259 hectares, in 1982 (As compared to 1975, this means a 7% territorial decrease) With the variety of cultivated species relatively often changing, the number of regularly cultivated species can be put at 30–40. Table 2 surveys the areal distribution of the major crops, except for such plants as paprika which are regarded mainly in Hungary as vegetable crops. The crop ratio underwent remarkable changes, over the span of 5 years. The largest area was given over to the cultivation of white mustard, followed by caraway, poppy, ergot, fennel, dill, ect.

Although even in those days in Hungary the individual farmers (auxiliary farming) played a significant role in the production of medicinal plants, now large scale, mechanized production systems have been elaborated for certain major crops, e.g. opium poppy, *Digitalis lanata*, ergot, white mustard, chamomile.

Table 1

Medicinal plants cultivated in Hungary, in 1982 (Authors' collective of OMFB, 1984)

Althaea officinalis	*Leuzea carthamoides*
Althaea rosea	*Linum usitatissimum*
Anethum graveolens	*Majorana hortensis*
Angelica archangelica	*Matricaria chamomilla*
Amsonia tabernaemontana	*Malva silvestris ssp. mauritiana*
Anthemis nobilis	*Melissa officinalis*
Artemisia abrotanum	*Mentha piperita*
Artemisia absinthium	*Mentha spicata*
Artemisia dracunculus	*Marrubium album*
Artemisia vulgaris	*Ocimum basilicum*
Brassica juncea	*Oenothera lamarckiana*
Calendula officinalis	*Papaver somniferum*
Carum carvi	*Pimpinella anisum*
Carthamus tinctorius	*Plantago altissima*
Claviceps purpurea	*Rosa canina*
Cnicus benedictus	*Ruta graveolens*
Coriandrum sativum	*Salvia officinalis*
Cucurbita pepo var. styriaca	*Salvia sclarea*
Digitalis lanata	*Satureja hortensis*
Dracocephalum moldavica	*Silybum marianum*
Fagopyron esculentum	*Sinapis alba*
Foeniculum vulgare	*Symphytum officinale*
Hyoscyamus niger	*Trigonella foenum-graecum*
Hyssopus officinalis	*Thymus serpyllum*
Lavandula angustifolia	*Thymus vulgaris*
Lavandula intermedia	*Valeriana officinalis*
Leonorus lanatus	*Verbascum phlomoides*
Lepidium sativum	
Levisticum officinale	

Table 2

The areal distribution of major cultivated medicinal plants, in Hungary

Crop	Area of cultivation (hectares)			
	1975	1980	1985	1987
Ergot	2630	4400	2100	1200
Poppy	2400	4500	6250	5700
White mustard	10900	18500	14600	9300
Dill	1430	2100	1600	2100
Lavender	610	300	250	270
Pfeffermint	150	300	350	400
Caraway	1310	450	6750	3500
Fennel	590	900	1200	2600
Marjoram	160	400	870	900
Chamomile	260	350	380	450
Sage	560	400	810	1300
Hop	640	690	620	520
Other species	1100	3100	6500	7850
Total	22740	36390	42280	36090

(Data by Jeszenszky—Bíró, M., 1988.)

Efficient production has been rendered possible by the efficient research and breeding activity of scientists and specialists working together in the field of both research and production of medicinal as well as aromatic plants.

The processing of medicinal plants

Figure 2 provides a simplified outline (flow diagram) of the channels through which medicinal plants can reach the phase of commercialization, with the ultimate stage of either export or domestic marketing. One of the major steps in this process concerns processing. The main aims of medicinal plant processing are to:

(a) provide drugs, drug mixtures for pharmacies. As an example, in the first half of 1983, the consumption of six main drugs (Chamomile flower, Rose hips, Linden flower, Fennel fruit, Anis seed, Sennae folium) amounted to some 151 tons. Table 3 provides a list of those uncultivated medicinal plants that are to be collected by the suppliers of Herbária, in 1989;

(b) produce essential oils both for the home consumption and exports. In 1982, the production of principal essential oils came to roughly 65,808 tons, while the value of exports amounted to some 867,000 Dollars (6);

(c) provide raw material for the pharmaceutical industry;

(d) provide aromas, colouring substances and spice extracts for the food, and canning industries as well as distilleries;

Table 3

List of principal wild-growing plants to be collected in 1989

Item	Quantity (kg)
Flowers:	
Blackthorn flower	3 000
Chamomile flower (fresh)	2 300 000
Chamomile flower (dry)	40 000
Elder flower (dry)	6 500
Elder flower (fresh)	200 000
False acacia flower	5 000
Hawthorn flower with leaves	15 000
Horchestnut flower	100
Larkspur flower	5 500
Lime flower	20 000
Linden flower	40 000
Melilot flower	500
Sunflower	2 000
Yarrow flower	3 000
Leaves:	
Black currant leaf	5 000
Common nettle leaf	40 000
Dandelion leaf	4 000
Mallow leaf	5 000
Pulmonary leaf	4 300
Herbs:	
Centaury herb	5 000
Golden rod herb	20 000
Melitot herb	10 000
Mother of Thyme herb	10 000
Mugwort herb	20 000
Shepherd's Purse herb	5 000
St. John's Wort	30 000
Yarrow herb	60 000
Roots:	
Burdock root	20 000
Great Celandine root	3 000
Nettle root	5 000
Seeds:	
Colchicum seeds	500
Juniper fruits	20 000
Rose hips (dry)	10 000
Rose hips (fresh)	100 000
Various items:	
Cherry stalks	3 000
Frangulae cortex	10 000
Mistletoe	55 000
Popular buds	1 000

(Data by HERBÁRIA Cooperative Enterprise)

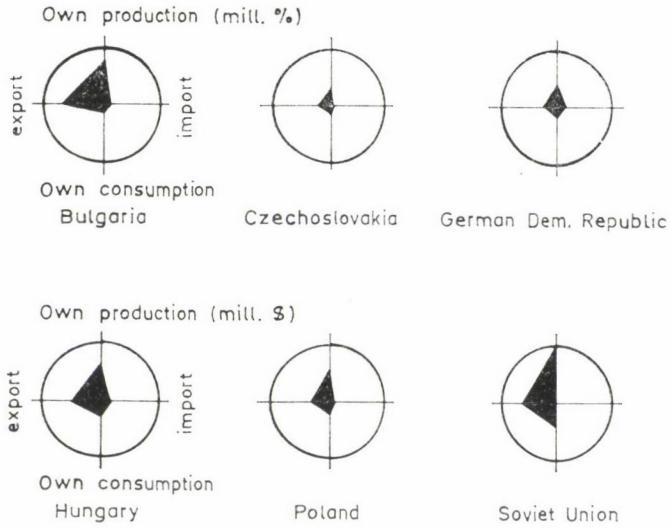

Fig. 1. Medicinal and aromatic plant production, consumption, export–import in certain socialist countries (Miszlay, 1975)

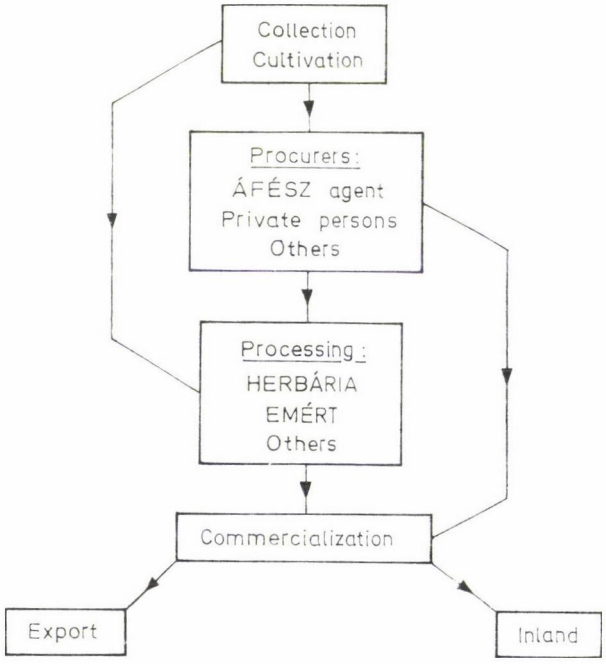

Fig. 2. Flow diagram for the gathering, processing and commerce of medicinal plants

Fig. 3. Changes in the structure of exports versus the main European importing countries (Data by Pharmatrade, 1989)

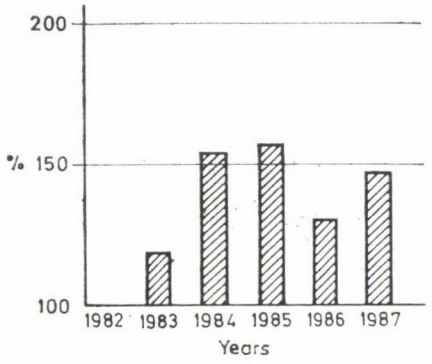

Fig. 4. Variations in the exports of Pharmatrade, between 1982–1987 (Data by Pharmatrade)

(e) meet the demands of the export markets. Since 1949 the medicinal plant export of Hungary has doubled in each decade. In 1987 it reached a level of 8–10 million Dollars, of which 70–80% was put into circulation by the PHARMATRADE Foreign Trade Company, Budapest (1). Changes in the structure of exports versus the principal spice-, medicinal plant, as well as essential oil purchasing countries of PHARMATRADE, in 1983 and 1987 (2) and certain variations in the exports, during the period between 1982–87 are summarized in Figure 3. and Figure 4., respectively. (A notable decline can be observed in 1986, probably owing to the Chernobyl disaster.)

Processing technologies are manifold, and vary from the traditional drying methods applied in auxilliary farming to the most sophisticated instant tea production technologies.

Conclusions

In summary, it can be stated that for a long time the high quality of drugs and the relatively low production costs have made the Hungarian medicinal plant products competitive.

During the last decade, however, a certain restructuring process has been taking place in the production, processing and also in the marketing of spice and medicinal crops. It is to be expected that this progressive development, also influenced by nature conservation, will increase the share of field production. The economic changes occurring in this country are likely to expand the share of small farms. By acquiring or elaborating modern processing technologies, the ultimate goal is to increase the share of both half-finished and finished goods. Being open to international co-operation, the Hungarian firms and enterprises together endeavour to combine the rich traditions of the past with the tempting possibilities of the present.

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Acta Agronomica Hungarica, 40 (1–2), pp. 201–215 (1991)

Reviews

SIGNIFICANCE OF THE ESSENTIALITY OF FLUORINE, MOLYBDENUM, VANADIUM, NICKEL, ARSENIC AND CADMIUM

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(Received: 17th January, 1989; accepted: 13th February, 1989)

Introduction

During the very long passage of the inorganic components of food, water and air through the fauna, they probably become part or activator of proteins, enzymes, hormones or tissues of the body.

Two groups of essential trace elements can be distinguished at present. The first category comprises trace elements which, in the case of an insufficient supply, lead to deficiency symptoms in animals and humans under the practical conditions. Fe, J, Cu, Mn, Zn, Co and Se belong to this group. Their essentiality was demonstrated in 1957 and all these belong to the group of classical trace elements. Such evidence in farm animals and game in controversial or not available in regard to F, Mo and Cr which belong to the second category and which are also classical trace elements. The same situation is true for all new trace elements (V, Si, Ni, As, Li, Pb, Cd). According to the present level of knowledge, sufficient amounts of these elements occur in nutrients as well as in water. Thus, no primary deficiency symptoms are to be expected in animals and humans (Anke et al. 1984a). Their essentiality is detected by means of semisynthetic rations.

The available data on the essentiality of F, Mo, V, Ni, As and Cd are summarized and critically assessed below.

Fluorine

As recently as 100 years ago, Erhardt (1874) recommended that children during the second dentition and children during the second dentition take fluorine pastilles. He recognized the densifying effect of fluorine on the dental

enamel in dogs, but this finding remained completely unnoticed at first. In the thirties, the correlation between a low degree of caries and the occurrence of dental fluorosis (mottled enamel) was demonstrated. Therefore, Cox (1939) proposed enriching the drinking water with 1.9 mg F/l. This was done in the USA from 1949 onwards, though there were and are opponents of this method.

The essentiality of fluorine has been repeatedly investigated. Schroeder et al. (1968) reported on a slightly decreased growth and a reduced life expectancy among mice fed on a fluoride-poor diet; Schwarz and Milne (1972) also found a limited influence of fluoride deficiency on the growth of rats. The negative effect of a fluoride-poor nutrition (0.1 to 0.3 mg F/kg) on the reproduction performance of mice observed by Messer et al. (1972, 1973) was not confirmed by Tao and Suttie (1976). An insufficient Cu and Fe supply in Messer's rations (1973) was assumed to be the reason for the different findings. Fluoride deficiency experiments repeated twice with growing, pregnant and lactating goats also showed no significant influence of the fluoride-poor nutrition with a semisynthetic ration which contained all necessary components.

Both the reproduction and growth performance of the goats with a fluoride-poor diet remained uninfluenced (Tables 1, 2). Apart from maternal mortality, they were not otherwise affected by fluoride deficiency. Further repetitions must clarify the influence of fluoride deficiency on the life expectancy of the fauna.

Table 1

The influence of fluorine deficiency on reproduction performance and life expectancy (n = 37; 13)

(Anke and Groppel 1988)

Parameter	Control animals	F-deficient animals	P
Success of first insemination (%)	70	85	0.05
Conception rate (%)	89	92	0.05
Services per gravidity	1.4	1.5	0.05
Abortions (%)	0	8	0.05
Kids per goat carrying to terms	1.3	1.2	0.05
Dead kids (%)	3	0	0.05
Dead mothers (%)	16	31	0.05

It can be summarized that no reliable evidence is available on the essentiality of fluorine. Most results obtained after fluoride supplementation are pharmacological effects; e.g. the positive results in the prophylaxis of dental caries and osteoporosis. Though a fluorine requirement for the biological

Table 2

The influence of fluorine deficiency on the pre- and post-natal development of kids (Anke and Groppel 1988)

Parameter (n)	Control kids		F-deficient kids		P	%[1]
	s	$\bar{x}$	$\bar{x}$	s		
1st day of life (kg) (42; 16)	0.63	2.7	2.8	0.59	0.05	104
91st day of life (kg) (31; 12)	2.2	15.6	15.4	4.2	0.05	99
168 experimental days (g/day) (15; 9)	12	107	102	26	0.05	95

[1] Control kids = 100%, deficient kids = x %

mineralization may exist, it has not yet been experimentally demonstrated. Therefore, Messer (1984) defined fluorine as "possibly essential".

Molybdenum

Molybdenum is a component of several enzymes in the flora and fauna (Table 3).

Table 3

Molybdenum-containing enzymes of different origin (Rajagopalan 1984)

Enzyme	Occurrence	Other prosthetic groups
Xanthin dehydrogenase	animal, plant, bacteria	FAD, Fe/S, MPT[1]
Aldehyde oxidase	animal	FAD, Fe/S, MPT
Nicotinic acid hydroxylase	bacteria	FAD, Fe/S, MPT, Se
Purine hydroxylase	A. nidulans	FAD, Fe/S, MPT
Sulfite oxidase	animal, plant, bacteria	Häm, MPT
Carbon monoxide dehydrogenase	bacteria	FAD, Fe/S, MPT
Assimilatory nitrate reductase	plants, microbes	FAD, Häm, MPT
Respiratory nitrate reductase	bacteria	Fe/S, MPT
Formic acid dehydrogenase	bacteria	Se, Fe/S, MPT
Nitrogenase	microbes	Fe/S

[1] Molybdopterin

Apart from nitrogenase, all Mo-depending enzymes contain the same molybdenum factor whose organic basis is a pterin ring with a side chain containing phosphate ester.

In spite of the clearly demonstrated existence of Mo in many enzymes, Mo deficiency in animals could only be induced by high doses of tungsten

taking an antagonistic effect. Tungsten can replace 30—40% of the Mo in the sulfite oxidase and, thus, inactivate it (Higgins et al. 1956, Johnson et al. 1974). Only the tenfold repetition of an Mo supply of < 24 μg/kg dry matter to growing, gravid and lactating goats via a semisynthetic ration resulted in a significantly reduced growth and reproduction performance in the Mo-deficient animals (Tables 4, 5).

Table 4

The influence of molybdenum deficiency on reproduction performance and life expectancy (Anke et al. 1983b)

Parameter	Control animals	Mo-deficient animals	P
Success of first insemination (%)	69	57	0.05
Conception rate (%)	83	71	0.05
Services per gravidity	1.5	1.9	0.05
Abortions (%)	1.4	15	0.01
Kids per goat carrying to terms	1.5	1.7	0.05
Dead kids (%)	3	28	0.001
Dead mothers (%)	25	61	0.001

Table 5

The influence of molybdenum deficiency on the pre- and post-natal development of kids (Anke et al. 1984b)

Parameter (n)	Control kids		Mo-deficient kids		P	%
	s	$\bar{x}$	$\bar{x}$	s		
1st day of life (kg) (90; 78)	0.78	3.1	2.9	9.77	0.05	94
91st day of life (kg) (50; 43)	4.2	19.6	15.3	3.9	0.001	78
168 experimental days (g/day) (56; 54)	19	92	67	25	0.05	73
168 experimental days (g/day) (5; 8)	41	131	97	13	0.05	74

The Mo-deficient goats had a significantly poor success of first insemination, needed more services per gravidity, aborted more foetuses and showed a greatly reduced life expectancy.

The influence of intrauterine Mo deficiency on the development of foetal body weight remained insignificant. The Mo-deficient goats, however, delivered significantly more kids with a live weight of < 2.0 kg than control goats (Anke et al. 1984b). The Mo-deficient kids weighed 22% less than the

control animals on the 91st day of life, i.e. at the end of the suckling period. Due to the increased intake of the semi-synthetic ration, the difference between the groups only became significant at the end of the suckling period. On the average, the milk of Mo-deficient goats contained double the Mo amount of the semi-synthetic ration (Anke et al. 1983b) (Table 6). The billy goats as well as the female goats gained 27 and 26% less weight than the control animals during the subsequent growth period.

Table 6

The influence of molybdenum deficiency on the molybdenum content of milk (μg/kg dry matter) (Anke et al. 1985a)

Stage of lactation	Control goats		Mo-deficient goats		P	%
	s	$\bar{x}$	$\bar{x}$	s		
Colostral milk (32; 23)	61	61	35	26	0.05	46
Nature milk (76; 48)	70	116	51	34	0.001	46

In humans, Mo deficiency was only reliably detected in a 24-year-old man after an 18-month parenteral nutrition. He suffered from headaches and lethargy, and excreted little sulfate via urine. The supplementation of 300 μg ammonium molybdate/day removed these symptoms (Abumrad et al. 1981).

The solutions applied for parenteral nutrition in the GDR contain between 0.3 and 8.0 μg Mo/l. In the case of long-term parenteral nutrition, 10 to 12 μg Mo/day might not cover the assumed Mo requirement of 25 μg/day in humans (Anke and Groppel 1988).

Furthermore, a genetic defect in humans can prevent the sulfite oxidation. In children, this defect leads to serious cerebral trauma, a disturbed mental development, the dislocation of eye lens, an increased renal sulfite and decreased sulfate excretion. The children are incapable of synthesizing the molybdopterin complex. Mo application does not overcome this problem (Duran et al. 1978, Johnson et al. 1980, Wadman et al. 1983).

The Mo requirement of the fauna is $<$ 100 μg/kg ration dry substance. There is no primary Mo deficiency since this requirement is always met (Anke et al. 1984b).

Vanadium

The effects of a V-poor nutrition were already investigated in rats and chicks at the beginning of the seventies. Chicks with 10 μg V/kg ration grew badly, with wing and tail feathers developing slowly (Hopkins and Mohr 1971). Rats with V-poor rations reacted to V supplementation with an improved

growth (Stradia 1971) and a better reproduction performance (Hopkins and Mohr 1974). The influence of V deficiency on the blood and the Fe metabolism of rats is controversial (Stradia 1971, Nielsen and Ollrich 1973, Williams 1973, Nielsen et al. 1983, Shuler and Nielsen 1984).

Since there were no definite results on the influence of a V-poor nutrition, the effects of V-deficiency (1 to 9 μg V/kg ration) were investigated in growing, gravid and lactating goats until the end of their lives. The experiments were repeated seven times. V deficiency decreased the success of the first insemination and significantly increased the number of services per gravidity as well as the abortion rate (Table 7). The number of kids per goat carrying to terms and the life expectancy of the mothers and their offspring were also significantly reduced by the V-poor nutrition.

Table 7

The influence of vanadium deficiency on reproduction performance and life expectancy (Anke et al. 1986a)

Parameter	Control animals	V-deficient animals	P
Success of first insemination (%)	77	57	0.001
Conception rate (%)	89	85	0.05
Services per gravidity	1.4	2.0	0.01
Abortions (%)	0	27	0.001
Kids per goat carrying to terms	1.5	1.1	0.001
Dead kids (%)	9	41	0.001
Dead mothers (%)	25	58	0.001

It was surprising that the pre- and post-natal development of the offspring remained completely unaffected by V deficiency (Table 8) whereas the milk production of V-deficient goats was significantly reduced by 9%. The

Table 8

The influence of vanadium deficiency on the pre- and post-natal development of kids (Anke et al. 1986b)

Parameter (n)	Control kids		V-deficient kids		P	%
	s	$\bar{x}$	$\bar{x}$	s		
1st day of life (kg) (93; 50)	0.80	2.86	2.87	0.79	0.05	100
91st day of life (kg) (48; 23)	4.0	17.2	16.4	4.6	0.05	95
168 experimental days (g/day) (53; 33)	34	92	92	34	0.05	100

offspring of the V-deficient goats frequently fell ill, and had skeletal damage of the forelegs. The goats suffered from pains in the joints and often changed their position in order to relieve the strain on their legs. Finally, the metatarsal joints thickened and deformations of the fore extremeties occurred.

The V content of the milk of control and V-deficient goats was extremely low (< 10 μg V/kg milk dry substance). Parts of the skeleton obviously reflected the V status of the fauna. No influence of V deficiency on the Fe status and the blood was registered. Though no V-dependent enzymes have been detected up to now in mammals and birds, V must probably be assigned to the essential elements (Anke et al. 1983a, 1985b, 1986a, 1988). The essentiality of V for humans was assumed, but not demonstrated (Hopkins and Mohr 1974).

It can be summarized that V deficiency has only an insignificant effect on growth whereas it reduces reproduction performance and life expectancy.

Nickel

The hydrogenase, carbon monoxide dehydrogenase and the methyl-coenzyme-M-reductase of several species of bacteria and the plant urease are Ni-dependent enzymes (Nielsen 1987). A Ni-poor nutrition (< 100 μg/kg ration dry matter in ruminants and < 50 μg Ni/kg in monogastric species) led to growth depressions in the fauna (Table 10), disturbed reproduction performances (Table 9) and a reduced life expectancy (Table 9) (Anke 1973, Anke et al. 1977, 1978b, 1983c, Schnegg and Kirchgessner 1975a, Nielsen et al. 1975, Spears et al. 1978, 1979).

Table 9

The influence of nickel deficiency on reproduction performance and life expectancy (Anke et al. 1984c)

Parameter	Control animals	N-deficient animals	*P*
Success of first insemination (%)	70	55	0.05
Conception rate (%)	83	71	0.05
Services per gravidity	1.4	1.9	0.05
Abortions (%)	1	9	0.001
Dead kids (%)	9	38	0.001
Dead mothers (%)	24	43	0.01

As a rule, chicks (Nielsen and Sauberlich 1970, 1975), mini-pigs (Anke 1973, Anke et al. 1974), goats (Anke et al. 1976b, 1980a) and rats (Nielsen et al. 1975, Schnegg and Kirchgessner 1975a, b) with an Ni-poor nutrition

Table 10

The influence of nickel deficiency on the prest and post-natal development of kids (Anke et al. 1983c)

Parameter	Control kids		Ni-deficient kids		P	%
	s	$\bar{x}$	$\bar{x}$	s		
1st day of life (kg)	0.77	3.1	2.8	0.80	0.05	90
91st day of life (kg)	4.2	19.1	16.3	3.8	0.001	85
168 experimental day (g/day)	33	99	88	42	0.05	89

suffered from lesions of the skin and had a shaggy coat of hair respectively. Pustules also occurred on the udders of goats (Anke et al. 1980a) and individual cases of dwarfism were observed as well (Anke et al. 1984c). Ni-deficient piglets renally excreted more calcium than control animals and had a lower Ca content in the skeleton (Anke 1973, Anke et al. 1974).

The Ni-deficient rats incorporated magnesium into the skeleton instead of calcium (Kirchgessner et al. 1980). The disturbed Ca metabolism induced a Zn depletion in goats (Anke et al. 1980a), piglets (Anke 1973, Anke et al. 1974) and rats (Schnegg and Kirchgessner 1976a). Ni deficiency leads to a reduced Zn incorporation into several organs and the milk, which was definitely demonstrated by ^{65}Zn in goats. It is due to a lower Zn absorption (Anke et al. 1984c). There are also interactions between Ni and Fe. Rats with a Ni-poor nutrition (Schnegg and Kirchgessner 1975b, 1976b, 1977a, 1977b, 1977c, Kirchgessner and Sohnegg 1980a, Nielsen and Myron 1980, Nielsen et al. 1979) and goats (Anke et al. 1980a, 1982) suffered from anaemia, which was caused by a reduced haemoglobin and haemotocrit content.

The microbiological digestion of ruminants, and of herbivores with appendiceal digestion, can be influenced by several Ni-dependent enzymes (e.g. urease), though urea is also decomposed by the ATP urea amidolase (Anke 1985). Ni-deficient animals had only 2% of the urease activity of control animals in their rumen content (Hennig et al. 1978). It was possible to increase the urease activity of the rumen content by supplying Ni to sheep, lambs and fattening cattle.

Ni deficiency also caused atrophy of testicles, a reduced production of permatozoa and Libido sexualis in adult billy goats (Anke et al. 1984c).

The Ni requirement of the fauna and of humans is < 500 μg Ni/kg dry matter, which may amount to < 100 μg/kg dry matter (Anke et al. 1984c) and is met by basic nutrition (Anke 1985, Anke et al. 1983d). Thus, no primary deficiency symptoms are to be expected.

It can be summarized that nickel is essential for the fauna.

Arsenic

According to Frieden (1984), the essentiality of arsenic is undisputed. Nielsen and Uthus (1984) wrote: "In 1975—1976 the first findings showing that arsenic is essential came from two laboratorias. The studies on arsenic essentiality apparently were done in each laboratory without knowledge that similar studies were being done in the other. As a result of these investigations signs of arsenic deprivation were described for chick, goat, mini-pig and rat. The signs of deficiency for mini-pigs and goats were reviewed by Anke et al. (1976a, 1978a, 1980b) and those for chicks and rats by Uthus et al. (1983)."

In spite of these findings, the functions of As in animals and humans are fairly unknown. The results obtained in As deficiency experiments repeated 12 times with growing, pregnant and lactating goats are summarized in Table 11 (Anke et al. 1986b). The As-poor nutrition (< 35 μg As/kg ration dry matter) induced a significantly poor success of the first insemination and a reduced conception rate in goats (Table 11). The As-deficient animals needed more services per gravidity and aborted significantly more foetuses.

Table 11

The influence of arsenic deficiency on reproduction performance and life expectancy (Anke et al. 1986b)

Parameter	Control animals	As-deficient animals	*P*
Success of first insemination (%)	75	57	0.01
Conception rate (%)	89	71	0.001
Services per gravidity	1.3	1.9	0.001
Kids per goat carrying to terms	1.4	1.4	0.05
Abortions (%)	0.8	15	0.001
Dead kids (%)	5.8	32	0.001
Dead mothers (%)	24	48	0.001

The life expectancy of the goats and their offspring was considerably reduced. Most goats died suddenly between the 17th and 35th day during the second lactation (Anke et al. 1980b, 1983e, 1985c, 1987b). No As-deficient goat survived the second gravidity. As-deficient goats reached an age of more than 6 years (Anke et al. 1983e). The ultrastructural changes induced by As deficiency mainly affect skeletal musculature and the membrane of the mitochondria of the heart (Schmidt et al. 1983, 1984). The influence of As deficiency on growth (Table 12) is less distinct. Only the kids showed a significantly reduced growth at the end of the lactation period. The milk of control and As-deficient goats was As-poor and contained less As than did the As-deficient rations in both groups. The offspring already accumulate large As amounts intra-uterinally on which they live during the suckling period.

Table 12

The influence of arsenic deficiency on the pre- and post-natal development of kids (Anke et al. 1986b)

Parameter	Control kids		As-deficient kids		P	%
	s	$\bar{x}$	$\bar{x}$	s		
1st day of life (kg)	0.76	4.0	2.8	0.74	0.05	93
91st day of life (kg)	4.5	18.6	16.2	4.3	0.01	87
168 experimental days (g/day)	33	90	75	27	0.05	83

As-deficient kids only had fractions of the As supplies of the control kids. As deficiency also reduced the amount of milk production of the goats, with the protein content being the same and the fat content of the milk being increased (Anke et al. 1987b).

The As requirement of the fauna was calculated to be < 50 μg/kg ration dry matter (Anke 1986). An As requirement of adult humans of 6 μg per 1000 kcal or 12 to 25 μg every day is deduced from the results of animal experiments (Nielsen 1984, Anke 1986c). The As demand of animals and humans is met by feedstuffs, foodstuffs and water in the German Democratic Republic (Krause 1986, 1988).

It is summarized that the available results point to the essentiality of arsenic, although no As-dependent enzymes have been found up to now. Further experiments must confirm the latter statement.

Cadmium

Cd belongs to the highly toxic elements. Due to its gradual accumulation in the kidney with increasing age. it is particularly dangerous for long-living species of animals (Friberg et al. 1974). In spite of its high toxicity and the existence of only few papers (Schwarz and Spallholz 1977, Anke et al. 1977) which deal with the essentiality of this element, Smith (1984) wrote: "Although these reports are not sufficient to establish definitively a specific function for cadmium, the metal is a good candidate for essentiality. The history of trace metal research should serve as a warning against taking too pessimistic a view on the possible essentiality of cadmium, because a number of other elements (notable Se) whose toxicity alone was initially appreciated have been subsequently proved to be essential."

More evidence for the essentiality of Cd is now available after the tenfold repetition of Cd deficiency experiments with growing, pregnant and lactating goats and their offspring.

Compared to control animals, the Cd-Poor nutrition (< 15 μg Cd/kg ration dry matter) reduced the success of the first insemination of the goats (300 μg Cd/kg ration dry matter) significantly (Table 13).

Table 13

The influence of cadmium deficiency on reproduction performance and life expectancy (Anke et al. 1987a)

Parameter	Control animals	Cd-deficient animals	*P*
Success of first insemination (%)	73	46	0.001
Conception rate (%)	85	72	0.05
Services per gravidity	1.2	2.2	0.001
Kids per goat carrying to terms	1.4	1.6	0.05
Abortions (%)	0	12	0.01
Dead kids (%)	8.0	43	0.001
Dead mothers (%)	30	41	0.05

The number of services as well as the abortion rate and the mortality of the mothers and their offspring increased significantly. The intra-uterinally As-depleted kids of several experimental years repeatedly proved to be very

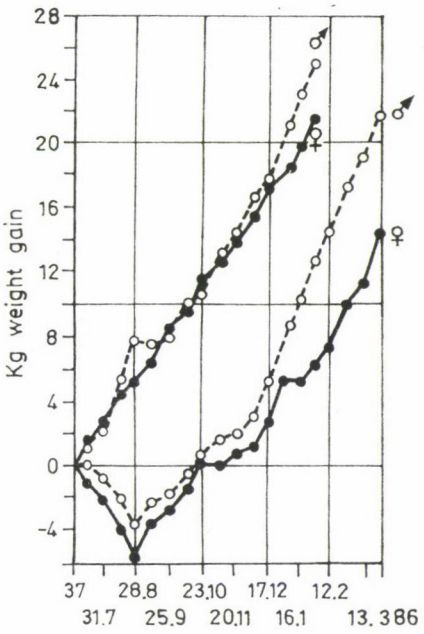

Fig. 1. Live weight gain of male and female control and Cd-deficient goats after weaning, and the feeding of the control ration to the Cd-deficient kids

phlegmatic; e.g. they were hardly able to keep their heads upright. In 1985, 9 kids of Cd-deficient animals were at our disposal for the first time. All kids showed an acute muscular asthenia about 6 weeks after being weaned. First they moved clumsily and stiffly. Later on, they became unable to move and could not stand. Finally, they were unable to raise their heads and died. After the death of 6 out of the 9 Cd-deficient kids, the 3 surviving animals which were also unable to move (2 males, 1 female) were fed on the ration of control goats with 300 μg Cd/kg. The animals slowly regained their mobility and gained weight (Fig. 1.). The same syndrome of muscular asthenia was registered in adult goats in 1987.

Cd deficiency did not have any significant effect on the growth of kids during suckling period (Table 14). There was also no significant growth depression in the kids after their weaning and the application of the Cd deficiency

Table 14

The influence of cadmium deficiency on the pre- and post-natal development of kids (Anke et al. 1987a)

Parameter	Control kids		Cd-deficient kids		P	%
	s	$\bar{x}$	$\bar{x}$	s		
1st day of life (kg)	0.76	3 0	2.8	0.74	0.05	93
91st day of life (kg(	4.5	18.6	16.2	4.3	0.05	87
168 experimental days (g/day)	33	90	75	27	0.05	83

diet; but the Cd-deficient goats produced 28% less milk than the control animals. Its fat content remained unaffected by the Cd deficiency whereas its protein content increased significantly. The ultrastructural analysis of the liver, the skeletal and cardiac muscles and of the kidneys showed that the Cd-deficient goats had generatively altered mitochondria in liver and kidneys. Christolysis and vesicular distensions were detectable. These findings indicate a reduced protein synthesis and an increased protein mobilization from the musculature in lactating goats (Anke et al. 1987a).

The essentiality of Cd can no longer be disregarded. The Cd requirement might amount to 20 μg/kg ration dry substance. A ration with 68 μg/kg did not meet this requirement (Anke et al. 1984d). Primary Cd deficiency symptoms are not to be expected in humans and animals (Masaoka et al. 1986) since the Cd content of nutritional material (Kronemann et al. 1982) is considerably higher than the postulated Cd requirement. The practical importance of Cd for the fauna and humans is in the field of toxicity (Kostial 1986).

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 217–236 (1991)

PROGRESS AND PROBLEMS OF BIOTECHNOLOGY IN THE SOYBEAN (*GLYCINE MAX* (L.) MERR.)

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(Received: 19th June 1989; accepted: 10th July 1989)

Introduction

The soybean (*Glycine max* (L.) Merr.) is one of those economically important plant species which are difficult to manipulate by the usual methods of plant tissue culture (Hildebrandt et al. 1986, Graybosch et al. 1987). As long as the in vitro cultures were used to study the physiological processes and the factors influencing them (Maróti 1976), it was sufficient to optimize the conditions of the callus- and cell suspension cultures. In respect to the soybean, this was already done in the sixties (Miller 1968, Gamborg et al. 1968).

The object of genetic interventions suitable to produce transgenic plants nowadays is the cultured plant cell, — tissue or -organ, from which through its totipotency — with the proper exogenous conditions provided — the whole plant can be regenerated (Heszky 1975). Elaborating a routine, reproducible plant regeneration system is a basic condition of applying the various biotechnological methods in higher plants.

Tissue culture research with the soybean began relatively early, yet the results have generally fallen behind those of investigations into tobacco-, rice- or, e.g., wheat tissue cultures. However, in the second half of the eighties considerable progress has been made, which calls for a systematic summarization of the results attained so far (Table 1).

Abbreviations

ABA: abscisic acid, BA: benzyladenine, B5: Gamborg et al. (1968) culture medium, CM: coconut milk, CPA: p-chlorophenoxy acetic acid, 2,4-D: 2,4-dichlorophenoxy acetic acid, IAA: indole-3-acetic acid, IBA: indole-butyric acid, KIN: kinetin, MS: Murashige-Skoog culture medium, NAA: naphthalene-acetic acid, 2,4,5-T: 2,4,5-trichlorophenoxy acetic acid.

Embryo culture

The *in vitro* development of isolated embryos is intended both to discover the processes taking place in the course of seed maturation, and to determine the conditions of *in vitro* embryogenesis. (Cutter and Bingham 1975, Obendorf et al. 1978, 1983, Eisenberg and Mascarenhas 1985)

Table 1

Induction of morphogenesis in soybean tissue cultures

	Explant	Culture medium + supplements	Result	References
Glycine max	hypocotyl	Gamborg (1966) and Miller (1965)'s medium + NAA + KIN + 2,4-D + CM and IAA	Root development: 35—90%; shoot regeneration 1—8%	Kimball and Bingham (1973)
Glycine max	leaf tissue	MS + NAA + KIN	Root development	Evans et al. (1976)
Glycine max *Glycine soja* *Glycine tabacina*	immature cotyledon	B5 + 2,4-D + KIN + IAA	Root development	Beversdorf and Bingham (1977)
Glycine max	seedlings cotyledon	Modified Phillips (1974) medium + 2,4-D + KIN	Shoot-bud differentiation (3% of the cultures)	Oswald et al. (1977)
Glycine max	cotyledonary node	Modified B5 + BA	Shoot and root development	Cheng et al. (1980)
Glycine max	anther	Modified B5 + GA_3 + IAA	Shoot and root differentiation	Guangchu et al. (1980)
Glycine max	stem and cotyledonary node	B5 + BA	Shoot and root development	Saka et al. (1980)
Glycine max	petiole, shoot tip, pod tissue, root	B5 + CM (protoplast) MS + 2,4-D + KIN (callus)	In protoplast culture root differentiation	Zieg and Outka (1980)
Glycine tomentella *Glycine canescens*	hypocotyl	MS + BA + NAA	Shoot regeneration shoot bud formation	Kameya and Widholm (1981)
Glycine max	shoot meristem and leaf primordia	MS salts B5 Vitamins	Intact plant regeneration	Kartha et al. (1981)
Glycine max *Glycine soja*	hypocotyl epicotyl	L2 Phillips and Collins medium (1979) + IAA + IBA, NAA or 2,4-D + BA or KIN or zeatin	Induction of somatic embryos and leaf development	Phillips and Collins (1981)
Glycine max	root tip	Modified B5 + CPA + BA and MS + IAA + BA	Root differentiation from protoplast culture	Xu et al. (1982)
Glycine max	hypocotyl and cotyledon	B5 + NAA 2,4-D MS + IAA + BA	Shoot development	Widholm and Rick (1983)
Glycine soja	embryo axis	MS + 2,4-D and reduced N-source MS + IBA + BA	Induction of somatic embryogenesis	Christianson et al. (1983)
Glycine soja *Glycine tabacina*			From protoplast embryo-like structure	Gamborg et al. (1983)

Glycine max	cotyledonary tissue	MS and B5 + 2,4-D	Somatic embryos in "torpedo" stage	Lippmann and Lippmann (1984)
Glycine canescencs	hypocotyl	B5 + IBA + BA	From protoplast plant regeneration	Newell and Luu (1985)
Glycine max	immature embryo cotyledon	MS + 2.4-D + B5 + ABA	Plant regeneration	Ranch et al. (1985)
Glycine max	immature embryo	MS + NAA + BA + KIN	Plant regeneration	Li et al. (1985)
Glycine max	immature cotyledon	MS salts + B5 vitamins + 2,4-D + NAA	Plant regeneration	Lazzeri et al. (1985)
Glycine max	immature cotyledon	MS + NAA + BA	Plant regeneration	Barwale et al. (1986)
Glycine max	anther	B5 + IAA + BA	Shoot regeneration	Jian (1986)
Glycine max	immature cotyledon and hypocotyl segments	L2 salts + B5 microelements and vitamins + 2,4-D + KIN	Shoot development	Kerns et al. (1986)
Glycine max	cotyledonary node	B5 + BA	Shoot development	Wright et al. (1986)
Glycine max	epicotyl	SH + 3-aminopyridine + BA + KIN	Shoot development	Wright et al. (1986a)
Glycine clandestina *Glycine canescens*	cotyledon		From protoplast plant regeneration	Hammatt et al. (1987)
Glycine max	immature cotyledon	MS + NAA	Production of soma clones	Barwale and Widholm (1987)
Glycine max	primary leaf	CS 23 + 2,4,5-T and B5 + BA	Shoot organogenesis	Wright et al. (1987)
Perennial *Glycine* varieties	seedling	B5 + BA + IBA	Plant regeneration	Hammatt et al. (1987)
Glycine max	immature embryo	KP8 medium (Kao 1977) + 2,4-D + NAA + zeatin MS-salts, B5 organic supplements + NAA + BA	Plant regeneration from protoplasts	Xu (1988)
Glycine max	cotyledon	B5 + BA	Plant transformed with Agrobacterium	Hinchee et al. (1988)

Hsu and Obendorf (1982) analysing the total protein-, lipid-, starch-, free amino acid-, DNA- and RNA contents of embryos developed under natural and artificial conditions, respectively, concluded that the natural processes could be simulated with in vitro conditions. Thompson et al. (1977) found that grains grown *in vivo* were highly similar to those grown *in vitro* as regards the synthesis of their reserve proteins, although the *in vitro* processes were quicker than the *in vivo* ones. Tilton and Russel (1984) isolated embryos of globular, heart- and cotyledon stage onto culture media containing various hormones. The most favourable germination and plant regeneration results were obtained with embryos past the heart stage in B5 medium, supplemented with 0.1 mg/l indole-butyric acid. Plants grown *in vitro* were smaller in size (with fewer and shorter internodes) and had smaller leaves than those obtained from seeds maturing under *in vivo* conditions. Raper and Patterson (1986) studied the temperature- and photoperiod requirements for *in vitro* embryos maturing through the trend of the fresh- and dry weight of fully developed embryos.

Tilton and Russel (1984) considered it important to establish the optimum conditions of an embryo culture, primarily to ensure the survival of hybrid embryos of soybean and wild *Glycine* species. Without the embryo culture, plants were earlier obtained *in vivo* from a mere 4.7 percent of the hybrid embryos (Newell and Hymowitz 1982). Broue et al. (1982) produced the hybrids *Glycine max.* × *G. tomentella* and *G. max.* × *G. canescens* by using embryo cultures, which meant the simultaneous introduction of embryo culture in the practice of plant breeding (Kurnik and Szabó 1987).

Morphogenesis from cultures of embryo origin

The importance of embryogenesis of organogenesis inducible in somatic cells of the developing embryo lies in the fact that, while with other plant species *in vitro* morphogenesis has been induced from the most diversified explants (anthers, mesophyll cells, segments of shoot apex, mesophyll protoplasts, mature or immature embryos), the isolation of the immature soybean embryo has given promising results.

Beversdorf and Bingham (1977) cultured immature cotyledons from 56 genotypes of the soybean species *Glycine max*, *G. soja* and *G. tabacina* on Blaydes (1966) culture medium, supplemented with 2 mg/l of each of 2,4-dichlorophenoxy-acetic acid (2,4-D) and naphthalene-acetic acid. After four weeks the cotyledons were transferred to a medium containing 0.01 mg/l 2,4-D and 0.3 mg/l kinetin, and subcultured for a further 28 days. During that period meristemic centres were formed in the callus. In the callus of Glycine max only roots developed from the embryo-like structures, but neither shoots nor

whole plants were obtained. Calli cultured longer than 13 months even lost their capability of rhizogenesis.

The plant — cell — plant system realizable in plant cell cultures works through somatic embryogenesis or through shoot morphogenesis. In the case of soybean the successful results obtained with other plant species (carrot, pea, manioka, etc.) could be reproduced neither by auxin induction and withdrawal, nor with a definite ratio of auxin and cytokinin.

The so-called morphologically competent culture — i.e. a culture capable of morphogenesis — produced by Christianson et al. (1983) from segments of embryo axis, using 2,4-D and a reduced nitrogen source, was in several respects an important step towards the development of a plant regeneration system for soybeans. These authors succeeded in producing embryoids in their experiments, and on the basis of their achievements they called attention to the normal and abnormal character of the developing somatic embryos, and to the importance of a balanced shoot- and root morphogenesis. Nevertheless, they did not provide a satisfactory method for *in vitro* regeneration of plants.

The somatic embryos induced by Lippmann and Lippmann (1984) from cotyledon tissues reached the "torpedo" stage of development, but plants could not be regenerated from them. Ranch et al. (1985) induced calli and somatic embryos from proembryos of globular and heart stage, using high concentrations (22.5 μM–430 μM) of 2,4-D. The developing embryos were cultured on B5 medium containing indole-butyric acid and abscisic acid; then the developed somatic embryos were germinated on B5- or MS-basal media containing 0.06 μM indole-butyric acid and 0.3 μM gibberrellic acid. After the development of the apical region, the plantlets were placed in a medium of B5 + 0.5 μM indole-butyric acid composition before planting in the soil. According to Ranch et al. (1985) the frequency of embryogenesis ranged between 20 and 90 percent, and plants with 2–4 nodes could be regenerated in 30 days from all of the mature somatic embryos. The authors put the successful plant regeneration at 20–60 percent, and considered the embryonic maturation to be the least efficient phase of the process. They also stressed the abnormalities of development occurring in the course of germination, a recurrent problem in their subsequent experiments.

Lazzeri et al. (1985) induced somatic embryogenesis from immature cotyledons on a MS basic medium, supplemented with B5 vitamins, using 2,4-D and naphthalene-acetic acid, and found that the 2,4-D, while more efficient than the naphthalene-acetic acid in inducing somatic embryogenesis, caused disorders of embryonic development.

Li et al. (1985) described a soybean regeneration method starting from a single cell. Pods containing immature embryos were subjected to cold treatment prior to isolation (20 minutes in liquid N_2), and from the embryos thus pretreated, calli were induced in liquid media. A cell suspension consisting

of individual cells was prepared by filtration, and from this the proembryos developed in a hanging drop culture. Then a 40-day incubation followed on MS-basal solid media containing various combinations of naphthalene-acetic acid, indole-3-acetic acid, 2,4-dichlorophenoxy-acetic acid, benzyladenine and kinetin. On the surface of the callus the somatic embryos appeared in the 0.2 mg/l benzyladenine and 0.01 mg/l indole-acetic acid treatments and in their combinations, respectively. The plant regeneration took place in a liquid culture. More than 1000 plants and a large number of differently developed somatic embryos per test tube were produced in 6 months.

This method that appears efficient from the standpoint of regeneration, however, has not been employed in recent experiments on the *in vitro* cell — plant system, probably because of its complicated nature.

Barwale et al. (1986a) used immature embryos of varieties selected on the basis of a so-called cotyledonary-node-shoot test (Barwale et al. 1986b). They induced somatic embryos with naphthalene-acetic acid instead of 2,4-D. A parallel induction of organogenesis was also successful. On the regenerating culture medium — with a 66 : 1 ratio combination of benzyladenine and naphthaleneacetic acid — they observed a shoot morphogenesis after four weeks. The efficiency of plant regeneration was 65–100 percent.

Hammatt and Davey (1986) started an embryo culture from proembryos of late globular or early cotyledonal stage of development. In the cultures the following development alternatives, depending on the culture medium, the genotype and the development level of the embryo, were observed:

— the embryos became callused;
— the embryos continued developing, then germinated;
— the embryos produced secondary embryoids (with 9–38 percent frequency).

In the adventitious embryos — as in the zygotic ones — germination was promoted by exsiccation (Hammatt and Davey 1986). The authors recommended the use of their method in producing and propagating sexual hybrids of wild *Glycine* species and soybean.

Lazzeri et al. (1987a) compared various concentrations of naphthaleneacetic acid and 2,4-D for their effect on somatic embryogenesis on the basis of the results of earlier plant regeneration experiments Lazzeri et al. (1985). They found that 2,4-D induced more somatic embryos than naphthaleneacetic acid of the same concentration, but embryo abnormalities (single cotyledon, fused embryo, neomorph) were fewer in the naphthalene-acetic acid treatment. The ratio of normal and abnormal embryos is important from the standpoint of plant regeneration, because intact plants can only be obtained from normal embryos. Lazzeri et al. (1987b) consider the induction to be the most critical phase of the whole process; in their opinion it depends on the applied type and concentration of auxin, and on the duration of the subculture.

The abscisic acid, which plays an important role in the *in vivo* development of the soybean embryo (Ackerson 1984a, b), either was ineffective or it inhibited the formation of embryoids, depending on the concentration used. Cytokinin (benzyladenine) in combination with auxin had a similar effect. Lazzeri et al. (1987b), in their paper concerning the effect of nutritive, physical and chemical factors on the somatic embryogenesis, indicated that the most critical point of the soybean plant regeneration, based on somatic embryogenesis, was to ensure the normal development of the viable embryos, which was scarcely influenced by the composition of the plant regenerating medium. With naphthalene-acetic acid induction that caused the least abnormality in their experiments, a comparison of the 1.5; 3.0; 6.0 and 12.0 percent sugar concentrations showed the 1.5 per cent concentration of glucose and 3.0 percent concentration of saccharose to be the most favourable for embryogenesis.

Recent examinations of experimental parameters of the induction of somatic embryos with normal morphology prove that increasing the efficiency of plant regeneration is still an important task (Hartweck et al. 1988, Lazzeri et al. 1988, Parrott et al. 1988), which may be promoted by analyses of the chemical composition of developing somatic embryos (Shoemaker and Hammond 1988).

Finer (1988) was able to increase the efficiency of somatic embryogenesis starting from immature embryo on a MS medium containing 40 mg/l 2,4-D and 6 percent sugar. By light microscope examinations of somatic embryos, he found that they originated from the epidermal, and subepidermal layers of immature cotyledons. Varying embrio morphology, callus formation and — above all — root differentiation are reported by Novák et al. (1987).

Two types of embryogenic callus (smooth and shiny, or rough and opaque) were observed on immature embryos, and pieces of hypocotyl and cotyledon used as initial material on a LS (Linsmaier and Skoog 1965) medium containing various concentrations of 2,4-D, IAA, BA and ABA (Ghazi et al. 1986). Plant regeneration could be induced in the smooth and shiny embryogenic callus with zeatin and GA_3 with 10–20 percent frequency.

The genotype dependence of somatic embryogenesis and plant regeneration was already determined by the first successful plant regeneration experiments (Ranch et al. 1985, Barwale et al. 1986). Komatsuda and Ohyama (1988) having examined 26 varieties, proved that the genotype made its effect felt in every important phase of the process: in the induction of embryogenesis, as well as in the development of the embryos and in their germinating ability. The regeneration of the Japanese commercial soybean varieties only exceptionally reached 3 percent (1 of 6 varieties). The highest plant regeneration rate of 10 percent was obtained with two selected embryogenic culture of *Glycine gracilis*.

Morphogenesis in cultures of various vegetative organs

The *in vitro* micropropagation of soybean, a plant species easy to propagate from seed, is of minor importance, but the meristem- or node cultures developed so far with the purpose of plant regeneration (Cheng et al. 1980, Saka et al. 1980, Kartha et al. 1981, Kameya and Widholm 1981) may be of use in propagating soybean lines produced by biotechnological methods (interspecific hybrids, protoplast regenerants, transformed plants, somatic hybrids).

Kimball and Bingham (1973) induced callus formation in hypocotyl segments using Gamborg (1966) and Miller (1965) media supplemented with 2,4-D naphthalene-acetic acid and kinetin. From hypocotyl segments placed onto culture media containing indole-3-acetic acid, coconut milk and kinetin calli rarely developed. The isolates swelled and from 35–90 percent of them, the root, from 1–8 percent, the shoot developed. The shoot morphogenesis usually was followed by the root generation. However, in cultures first regenerating the root, the shoot did not develop. Oswald et al. (1977) induced calli from medial sections of the cotyledons of very young seedlings on a Phillips (1974) culture medium supplemented with 2,4-D and 2,4,5-T. From 3 percent of the callus cultures, shoot buds were induced with 2,4-D and kinetin. On the other hand, at the beginning of the eighties Kameya and Widholm (1981) obtained neither adventitious nor callus origin root- or shoot differentiation from hypocotyl cultures of various *Glycine* species.

From the callus formed on a solid L2 (Phillips and Collins 1979) medium from hypocotyl and epicotyl segments, a suspension culture was established by Phillips and Collins (1981) with *Glycine max* and *Glycine soja* species. From the suspension the cells were re-transferred onto a solid medium after 1–18 months. The L2 medium was supplemented with IAA, indole-butyric acid, α-NAA or 2,4-D for the sake of comparison effect of auxin The cytokinins adenine sulphate, kinetin, benzyladenine and zeatin were added to the culture medium, which was supplemented with antiauxins (2,3,5-tri-iodo--benzoic acid, abscisic acid, glutathon, gibberellic acid) and a gibberellin biosynthesis inhibitor (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylate-methyl-chloride). Somatic embryos were successfully induced from all soybean genotypes examined. The number of embryos and their development stage (globular, heart) depended on the age of the cultures, and the quality and concentration of the regulators. On a solid culture medium (L2 + 0.45 — 2.25 μM 2,4-D) even shoot development was observed. The "shoots" thus produced grew more leaves after the first two ones, but did not develop into whole plants. The antiauxins and the gibberellin synthesis inhibitors, while stimulating the root development, had no effect on shoot organogenesis.

Evans et al. (1976) induced root differentiation from leaf tissue callus on a MS culture medium, supplemented with NAA and kinetin. Chang et al. (1980) succeeded in regenerating soybean plants from cotyldedonal segments with adventitious shoot differentiations on a B5 culture medium, supplemented with indole-butyric acid and benzyladenine.

Widholm and Rick (1983) were able to produce shoot development in calli induced from hypocotyl- and cotyledonal segments of *Glycine canescens*.

Attention should be paid to the experiments in which the capability of the cotyledon node for multiple shoot formation was studied in order to select genotypes in which the *in vitro* shoot morphogenesis is easier to induce and maintain. Barwale et al. (1986b) compared 155 *Glycine max* and 13 *Glycine soja* genotypes. After 4 weeks of incubation on a B5 culture medium, in the cotyledon node of the plantlets, shoots varying in number depending on the genotype, developed in response to 1–5 μM benzyladenine. In the case of the *Glycine max* varieties, the number of shoots ranged between 2 and 11 on a culture medium containing 1 μM BA, and between 1 and 12 in response to 5 μM BA.

From the genotypes 14 were selected (Kerns et al. 1986) and placed in three groups on the basis of average shoot production: either good (9 shoots or more), medium (5–8 shoots) or poor (4 shoots or less). Hypocotyl segments of plantlets cultured under sterile conditions, or the abaxial halves of cotyledons, were placed onto a culture medium containing L2 salts (Phillips and Collins 1979), B5 microelements, vitamins, as well as, 0.2 mg/l kinetin and 0.4 mg/l 2,4-D. From a callus developed in darkness during 35–40 days, suspension cultures were established in which 0.4 mg/l 2,4-D was the only regulator. The development of the somatic embryos was checked every week, and it was found that the embryogenesis and the capacity of the multiple shoot formation were in correlation.

Bathia et al. (1985) induced adventitious shoots by dropping benzyladenine of 0.1 mg/l concentration onto cotyledons of several-day-old plantlets, after removing the embryo axis.

Wright et al. (1986) succeeded in attaining regeneration from cotyledon node explants through de novo shoot organogenesis, proven by histological examinations. Similar results were reported by Wright et al. (1987a) for epicotyls cultured on a SH medium (Schenk and Hildebrandt 1972), supplemented with 3-amino-pyridine, benzyladenine and kinetin.

Extremely promising is another plant regeneration experiment also carried out by Wright et al. (1987b). After cutting up primary leaves of soybean plantlets longitudinally (along the midrib), they placed the segments onto a CS23 culture medium (Reynolds et al. 1982) containing 2,4,5-trichloro-phenoxy acetic acid, glutamine and adenin sulphate to produce callus. After four weeks of incubation, the shoot organogenesis was induced in a B5 medium containing 5 μM benzyladenine. They were then rooted on a hormone-free B5 or MS

medium, whereby *in vitro* plants were obtained with a frequency of 40–60 percent. Hammatt et al. (1987) succeeded in inducing shoot organogenesis from calli formed on plantlets of *Glycine* species (*G. canescens*, *G. falcata*, *G. latifolia*, *G. tabacina*, *G. tomentella*).

Somaclonal variation

Barwale and Widholm (1987) throw a qualitatively new light upon the somatic embryogenesis. Studying the factors influencing the plant regeneration was no longer the aim in their work; tissue culture served to increase the genetic variability. From 10 genotypes 263 plants were regenerated, 153 of which were studied over several generations, and among them chlorophyll deficient, totally or partially sterile, wrinkled-leaved, twin-seeded, dwarf variants were found.

A small extent of somaclonal variability was also observed by Graybosch et al. (1987) in field experiments with soybean plants regenerated with the method of Wright et al. (1986).

Important agronomical properties (nodulation, yielding ability, resistance to cold and disease) were studied by Ancelet et al. (1988) in plants regenerated from immature embryos of Maple Arrow and Hodgson varieties.

Androgenesis in vitro

Ivers et al. (1974), in their experiment to establish soybean anther cultures, compared the development phases of the flower bud to the stages of anther- and pollen development, and succeeded in indlucing calli from which, however, neither embryos nor shoots developed.

The first haploid (2n=20) plant successfully produced in soybean anther culture was reported by Guangchu et al. (1980) of which an account is given in the summary written by Hildebrand et al. (1986) on soybean. The anthers containing mononuclear pollen grains developed calli on a modified B5 culture medium. On a B5 medium containing gibberellic acid and IAA, shoots and roots differentiated from the callus. In anther culture started from 85 varieties and from the F_1–F_5 generations derived by crossing them, Jian et al. (1986) found a genotype which regenerated shoot with a 1 percent frequency. In the case of the other genotypes, plants could not be regenerated from the large number of embryos developing from the pollen callus.

Callus culture

The callus and cell suspension cultures of soybean are important objects for studying the processes of metabolism, physiology. The examination of metabolism and of ecological, nutritional and hormonal factors acting on the cells has been one of the purposes of using in vitro cultures, as Haberlandt stated at the very beginning of the history of plant tissue culture (Maróti 1976). Related with the callus cultures, a part of the investigations was intended to produce the culture itself, analysing the physical and chemical factors influencing the callus induction and proliferation. The first step in these analyses was the elaboration of the Miller (1961) culture medium, and then Gamborg's B5 medium (Gamborg et al. 1968), which took the nutrient demand of the root cells of soybean into consideration. The B5 culture medium has ever since been widely used, not only in suspension cultures, but also in solid cultures.

Hemphill and Venketeswaran (1977) carried out experiments to determine the conditions required for the growth of green and chlorophyll-free soybean calli. Schwenk (1981) produced a soybean callus culture from the mesophyll of primary leaves by mechanical isolation.

The cytokinin biotest based on the growth of a soybean callus was first used by Miller (1968, 1972); since then this method has also been introduced into laboratory practices (Reinert and Yeoman 1982).

The growth of calli developing from cotyledon and hypocotyl segments (Newton et al. 1980) is greatly influenced by the zeatin, the synthetic cytokinins, and by thymidine analogues (Wang 1979, Van Staden 1979, Van Staden and Hutton 1982, Palni et al. 1984, Forsyth and Van Staden 1986).

The soybean callus culture is highly important method in the field of application of tissue culture; in plant breeding and mutant selection for herbicide, pest and pathogene resistance.

Gray et al. (1986) inoculated pathogenic and non-pathogenic filtrates of *Phialophora gregata*, the pathogen of brown stem rot, into calli of genotypes susceptible and resistant to the disease. The experiment was suitable to determine the callus level resistance of soybean, on the one hand, and the pathogeneticity of the fungus isolate, on the other. In contrast to the soybean callus the tobacco callus gave no response to infection by *Phialophora gregata*. By callus selection ethionine-resistant soybean lines were produced by Madison and Thompson (1988). Among the lines selected for ethyionine, types were found that contained free methionine at higher concentrations than were in the wild cell lines (Greenberg et al. 1988). The examination of their homoserine kinase- and threonine synthase activity was intended to answer the questions concerning the methionine synthesis.

Suspension culture

The suspension and callus cultures of soybean are fairly equal in importance in regard to their application:

— determination of the nutrient demands of cell cultures (Gamborg et al. 1968)
— synchronization of cell division (Constable et al. 1974)
— determination of metabolites, precursors (Moore 1977, Poulton and Krauer 1977) and tracing of synthetic processes (Bevan and Northcote 1981, Eisenberg and Mascarenhas 1985, Herber et al. 1988)
— selection of nutritional variants (e.g. selection of slowly and rapidly growing soybean lines on maltose as a carbon source (Limberg et al. 1987)
— study of possibilities for mutant isolation (Polacco 1979), development of herbicide resistance (Hyung-Yul Cho et al. 1986, Chowdhury et al. 1986)
— comparison of dry matter synthesis and photosynthetic activity in chlorophyll-containing and chlorophyll-free cell suspensions (Klerk–Kiebert et al. 1981)
— measurment of enzime activity and study on enzime expression (Chiu and Shargool, 1979; Matthews and Widholm, 1979; Havir, 1981; Kochs et al. 1987)
— response to stress effects (Leguay et al. 1988).

Owing to the outstanding role of auxins in the induction of somatic embryogenesis, those suspension cultures in which the 2,4-D metabolism of soybean cells are studied deserve special attention. Christianson (1985) terms those cells embryogenic which to a definite stimulus, namely the reduction of the level of synthetic auxin, respond with somatic embryogenesis.

Scheel and Sandermann (1981) followed the metabolism of radioactive (2–^{14}C) and cyclically labelled (^{14}C) 2,4-dichlorophenoxy-acetic acid in soybean and wheat cell suspensions. Through 60 and 25 cell cycles of the soybean and wheat suspensions, respectively, maintained for 8 years, they determined the quantity of the demonstrable labelled 2,4-D. The main metabolite fraction of the soybean suspension consisted of amino acid-2,4-D-, while that of the wheat suspension was composed of D-glucoside-2,4-D conjugates. In both suspensions new, unidentified water soluble metabolite and conjugate, formed with low molecular weight protein, were also detected. By further investigations Scheel and Sandermann (1981) indicated that a part of the 2,4-D was covalently linked with lignin, which is a possible method of cell detoxification.

Steward (1958) produced somatic embryos from a carrot cell suspension by decreasing the concentration of 2,4-D. As previously mentioned, this method has not produced a similar result from soybean. Montague et al. (1981a,b) had every reason to suppose that a comparison of carrot- and soybean cell suspen-

sions for 2,4-D metabolism would help in understanding the phenomenon. The larger proportion of the labelled 2,4-D was detectable in the form of amino acid conjugates in the soybean cells, and in a free state in the carrot suspension. In a 2,4-D-free medium the labelled compound was released at different rates from the soybean- and carrot cells previously exposed to the action of 2,4-D: from carrot cells at a faster rate than from soybean cells. The difference between the two species is reflected by the fact that 72 hours after the inoculation with 2,4-D, nearly 90 per cent of the 2,4-D was present in a free state in the carrot cells, while in the soybean cells 34 per cent of it was found free and 58 per cent in an amino acid conjugate. The retention of 2,4-D due to its incorporation into amino acid conjugates may prevent the morphogenesis in cell- and callus cultures of soybean.

The cytokinins inhibit the conjugation between 2,4-D and amino acids (Montague et al. 1981b). The treatment of soybean cells with suitable concentrations of kinetin resulted in a 2,4-D metabolism resembling the one in the embryogenic carrot cells. Nevertheless, morphogenesis could not be observed in these soybean suspensions; that is, the 2,4-D retention must be a sole reason for the unfavourable phenomenon. In the case of woody plants the inhibition of somatic emdryogenesis is explained by the formation and toxic effect of polyamines too (Litz and Schafer 1986).

Protoplast culture

Fowke et al. (1974) were the first to study the mitosis of protoplasts and cells isolated from cell suspension. Hanke and Northcote (1974) examined the cell-wall regeneration of protoplasts produced from a soybean callus. Pectine present in the culture medium did not incorporate into the cell-wall during 40 hours of culturing. The biosynthesis of polysaccharides was investigated by Klein et al. (1981) in an early phase of cell-wall regeneration in protoplasts obtained from a cell suspension.

Protoplasts were isolated from various tissues of soybean: from root nodule (Gresshoff and Rolfe 1978), roots of seedlings (Ku et al. 1982), mesophyll (Schwenk et al. 1981), cotylendons of immature or mature seeds (Kao et al. 1981, Lu et al. 1983), and from a suspension of hypocotyl origin (Chowdhury and Widholm 1985).

Zieg and Outka (1980) treated petioles, cotyledons, shoot tips and pods with enzyme mixtures. The best result was obtained from pod tissue (5×10^6 protoplasts (ml). The protoplasts regenerated their cell-walls began dividing and formed calli from which roots may even develop under suitable conditions. Xu et al. (1982) only observed rhizogenesis in protoplast cultures of root tip origin.

Lu et al. (1983) produced protoplast populations capable of division by treating seedlings and immature seeds with enzymes. The new methods of protoplast culture which promoted the development of a plant — protoplast — plant system were described by Tricoli et al. (1986). A plant regeneration from protoplasts of wild soybean species was first solved by Newell and Lu (1985), from hypocotyl protoplasts of *Glycine canescens* they obtained calli capable of morphogenesis, though with low frequency, from which they regenerated whole plants. From *Glycine clandestina* and *G. canescens* Hammatt et al. (1987) produced *in vitro* plants of protoplast origin. Gamborg et al. (1983) obtained embryo-like structures from protoplasts of the species *Glycine soja* and *Glycine tabacina*.

Wei and Xu (1988) isolated protoplasts from immature embryos of commercial soybean varieties using Onozuka R 10, hemicellulase and macerozyme 10 enzyme mixtures. In the KP8 culture medium (Kao 1977) containing 0.2 mg/l 2,4-D, 1 mg/l NAA and 0.5 mg zeatin the protoplasts kept in darkness began dividing and formed calli. From the calli on a regenerative culture medium (MS salts and B5 supplements+0.5–2 mg/l 2,4-D or NAA+0.1–0.5 mg/l BA) a compact and nodular callus was formed which, on a culture medium containing 0.15 mg/l NAA, 0.5 mg/l BA, kinetin and zeatin as well as 500 mg/l caseine hydrolysate, developed shoots. From 4 varieties they regenerated a total of 87 plants.

Somatic hybrids

Simultaneously with the establishment of protoplast cultures, a fusion technique was applied to soybean tissue cultures. The spontaneous interfusion of soybean protoplasts, as well as the formation and mitotic behaviour of multinuclear protoplasts, were studied by Miller et al. as early as 1971.

Fusion was brought about between protoplasts of barley, pea, tobacco species, colchicum, white clover, rice, lucerne and soybean (Kao and Michayluk 1974, Constabel et al. 1976, Fowke et al. 1976, Niizeki and Kita 1981, Dudits 1982, Kao and Saleem 1986). The products of fusion divided and remained alive for some days or occasionally for several months.

Studying chromosomes of *Nicotiana glauca* — soybean heterokaryons Kao (1977) observed anaphase bridges, formation of extremely long chromosomes, ring chromosomes and fragments during the process of division.

Despite some aberrations, chromosomes of both partners could be found in the hybrid cells even after 6 months.

In the pattern of somatic hybrids of *Nicotiana* — soybean Wetter (1977) still detected bands characteristic for both parents after eight months.

Genetic engineering

The uptake of foreign plasmid-DNA was accomplished at the beginning of the eighties with soybean protoplasts isolated from synchronized cell suspensions (Cress 1982).

To produce transformed plant cells, permeable protoplasts are required for introducing the donor DNA molecule. The necessary permeability can be achieved either by chemical (Saleem and Cutler 1986) or by physical (Cutler and Saleem 1987) methods.

Lin et al. (1987) introduced plasmid-DNA into protoplasts freshly isolated from immature embryo cotyledons using polyethylene-glycol and -electrophoresis techniques. The presence of stable transformates was suggested by the fact that the activity of chloramphenicol-acetyl-transferase (CAT) originating from the plasmid could be demonstrated even in a 40-day callus culture.

Christou et al. (1987) achieved a root development from the soybean callus formed from stable transformed cells (kamanicin resistant, possessing aminoglycosid-3-phosphotransferase activity) obtained by electroporation.

In a new method of introducing foreign DNA, tungsten or gold particles are coated with DNA molecules or plasmids, which permeate the cell-wall. In this way the callus level transformation, even of plant species difficult to regenerate from protoplasts, is possible. Christou et al. (1988) bombarded 4–8 mm zygotic soybean embryo with gold particles of 1–5 μm diameter coated with plasmid (pCMC 1022). Immediately after this treatment with the microparticles, they carried out a protoplast isolation. By neomycin phosphotransferase (NPT) activity determination and Southern-hybridization, they demonstrated that the plasmid DNA incorporated in the soybean genom. They failed to regenerate plants from protoplasts isolated from the transformed soybean cells.

Of the use of *Agrobacterium tumefaciens* as a transformation vector in the suspensions of soybean protoplasts regenerating cell-walls, a description was supplied by Baldes et al. (1987). They proved the expression of the T-DNA gene by the kanamycin resistance of the cells and with molecular methods, and found that the soybean belonged to those plant species that are easy to transform but difficult to regenerate.

Hinchee et al. (1988) produced transgenic soybean plants by *Agrobacterium mediation*. The odd thing about the method is that the transformation was carried out in differentiated tissue on the cotyledon, and not in cell-, callus- or protoplast cultures. From cotyledons infected with *Agrobacterium* they obtained transformed plants by shoot multiplication with BA; 3–4 months after the inoculation, the transformed plants possessed kanamycin (NPT) or glyphosate resistance (EPSP) and glucoronidase (GUS) activity as the proof of the gene insertion (6 per cent of the plants examined). In the progeny of these plants a 3:1 Mendelian segregation ratio was discovered for glyphosate tolerance or kanamycin resistance.

Summary

Owing to its economic importance the soybean has been an object of plant tissue culturing since the sixties, The efforts to develop a plant-cell-plant system achieved the first successes of plant regeneration in 1985. The maíor laboratories, however, are still striving to increase the efficiency of regeneration. It is a promising fact that the problem of transgenic production of soybean has been solved by carrying out tissue-level rather than cell-evel transformations.

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 237–251 (1991)

ASPECTS OF THE *IN VITRO* TECHNOLOGY OF APPLE (*MALUS* SP.)

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(Received: 2th April 1990; accepted: 3th June, 1990)

Introduction

The apple, *Malus domestica* (*Rosaceae*), is one of the most important of fruit species in the world. It is widely adapted and is grown in temperate regions that have a distinct cold period (37).

Malus pumila (*M. sylvestris*, *M. communis*, *Pyrus malus*) embraces most of the cultivated fruit apples (36). Wild or crab apples, *Malus sylvestris* were widespread many thousands of years ago in the forests of Asia minor, the Caucasus, Persia, Armenia and Kurdistan and occurred throughout most countries of Europe. Several *Malus* species, which are still found in the apple forests of the Caucasus, played an important part in giving rise both to our cultivated forms and the types used widely as rootstocks (65).

World-wide apple production amounts to some 40,923 million tons annually. Europe is the leading apple growing-continent. France 2,650,000, Italy 2,080,000 and Germany FR 2,180,000 are the leading European apple producing countries (FAO, Yearbook, 1987).

In vivo-propagation methods

Apple trees for commercial orchards are propagated by grafting selected cultivars onto seedling rootstocks raised either by stooling/layering, or propagated clonally (e.g., dwarfing rootstocks) (10, 42).

T-budding as well as chip budding are frequently used methods, though they demand expensive nursery facilities.

Root-grafting is usually used as whole-root grafts. Propagation of cultivars by hardwood cuttings, with the exception of certain clonal rootstocks, is seldom used (25).

Softwood cuttings can be rooted under mist, though this method is not used commercially (25). Mound layering is also a common method of obtaining clonal rootstocks for apple (26, 88).

The difficulty of propagating the scion cultivars by traditional methods has precluded the production of self-rooted trees. Clonal rootstocks have therefore been regarded as essential for ease of propagation and control of tree

size. The possibility of self-rooted dwarf apple trees now appears to merit reconsiderations (41, 43).

However, the propagation and establishment of the rootstock, budding or grafting with the scion and growing-on to form a 1- or 2-year old tree in the nursery are lengthy and increasingly expensive procedure. Thus, the methods of reducing tree costs have become of considerably important (83). As both scion cultivars (40) and rootstocks (39) readily respond to *in vitro* techniques, these methods have gained increasing importance.

Role of *in vitro* methods in the propagation of apple

The apple is one of the first woody plants for which successful results were obtained with micropropagation techniques (14, 59, 61, 94). In 1971, Abbott obtained callus from stem segments and from shoot apices. However, it was not until 1976 that ABBOTT and WHITELEY (1) succeeded in regenerating a complete plant from shoot apices taken from green-house-grown seedlings (1). Since then, others have described meristem and shoot apical culture of several rootstocks, such as Northern Spy (35), M.7 (87), M.26 (11, 39, 12, 58) and EMLA-27 (9, 58, 60), MM 106 (5, 13) and MM.104 (13, 20, 60).

The micropropagation of both rootstocks and fruiting cultivars is possible with careful attention to the requirements of the various stages of development.

Cultures of vegetative plant parts

Meristem-tip culture

Meristem-tip culture has recently become an important technique, particularly with vegetatively-propagated species, e.g. apples. This technique, valuable for rapid clonal multiplication of selected cultivars, is also used for:

— producing virus-free plants,

— international exchange of disease-free plants,

— breeding functions, e.g. germplasm (49).

The principle of the method is that apple meristem tips are dissected from lateral buds of either active or dormant shoots and are established on a medium that includes the Lepoivre macronutrients, Murashige and Skoog (MS) micronutrients (56) and the vitamins of Walkey (80). Once the explant shows leaf expansion and shoot elongation, it is transferred to a proliferation medium that contains higher concentrations of minerals and growth regulators. During proliferation, the tissue is subdivided and transferred to a fresh medium at specific intervals, usually 3–4 weeks. Cultures are subdivided numerous times to provide propagules for rooting and 1–2 cm long shoots are collected from proliferating cultures for this purpose (6).

Shoot-tip culture

The shoot-tip culture is a widely used tissue culture method also for recovering pathogen-free plants. The explants range in size from 0.1 mm to 2 mm long stem tips. The explant may be all or in part an apical or lateral growing point of a stem or a stem section with several nodes. After surface sterilization the apical 3–5 mm of the shoot tip is dissected and placed vertically on LS medium supplemented with growth regulators (5 μM BAP, 1 μM IBA and 1 μM PG). Following shoot proliferation rooting can be readily induced with IBA, though salt concentration and physical properties of the medium also influence both root initiation and growth. Rooted plantlets are transferred either to vermiculite: perlite (1:1) or to peatmoss: coarse sand (1:3), and kept in a shaded glasshouse under mist (5 sec every 15 min.) for 2 weeks and ther transferred to the greenhouse bench (17, 28).

In vitro micrografting

The primary use of this technique is to produce virus-, viroid-, or mycoplasma-free woody plant material, especially in the case of species or varieties that do not easily regenerate shoots/roots through shoot-tip culture. The method has been successfully used to recover pathogen-free *Malus* and *Prunus* plants (25).

In practice, the shoot-tip is grafted onto an *in vitro* grown rootstock seedling. The rootstock must be free of infection which means that the pathogen is not transmitted through the seed. The seeds are germinated aseptically and grafting is also done aseptically when the seedling is at least 2–3 weeks old (25, 77). The method seems to have relevant advantages in the case of the apple stem grooving virus (SGV), an apple disease that is not eliminated by thermotherapy. Most cultivars of apple infected with SGV show no symptoms. So, the in vitro micrografting of apple shoot tips is suggested as a technique to produce virus-free plants from some explants of infected cultivars, cultured shoots of which are difficult to root (27).

Callus culture

An apple callus can usually be produced from any differentiated structure (e.g., leaf, stem, root). Once produced, the callus can be grown either as large, multicellular masses on solid media, or as small cell aggregates in rotated liquid media. The undifferentiated callus can be stimulated into producing differential structures which eventually develop into a shoot (55, 23).

The callus induction from stem segments of *in vitro* cultured apple shoots has been reported a possibility in the case of the cultivar "Akero". The

MS medium (56) contained different concentrations of macronutrients, auxin (IBA) and cytokinin (BAP). Callus induction took place when using two concentrations of macro-nutrients, full strength and half strength in combination with 0, 0.1, 1.5 mg/l of IBA and BAP. Six weeks after transferring the callus to media without IBA, green nodular cell aggregates were found in a number of calli. After transferring the callus to fresh medium and a further 7 weeks of growth, farther shoots were formed from previously observed nodules (20, 23, 67, 82).

Organogenesis in callus of stem and leaf origin

The callus formation from stem internodes of apple rootstocks "M.9", "M.25", "M.26", "M.27" was initiated on 4 NAA-based media (2–10 mg/l). Transfer to media lacking NAA allowed the shoot regeneration with the exception of the varieties "M.9" and "M.26". Organogenesis was also obtained from leaf discs of "M.27" employing BAP at 5 mg/l and 2,4-D at 0.1 mg/l. The regenerated shoots were subsequently multiplied and rooted. Organogenesis also occurred in "M.26" from small (1–2 mm), green, compact, embrioid-like structures derived from stem and leaf surfaces of excised axillary shoots. These structures differentiated into shoots at a low frequency ($<1\%$) on media containing BAP (1 mg/l) and IBA (0.1 mg/l) and could also be micropropagated by subsequent axillary shoot proliferation (34, 86).

Plant regeneration from callus of root origin

Callus was initiated *in vitro* from the roots of intact micropropagated plantlets of the apple rootstock "M.25" and from the excised roots of such plantlets. Shoots originating directly from the callus or from the embrioid-like structures have been dissected to produce plants for evaluation in the field (42).

Protoplast culture

The protoplast technology might be useful for the solution of practical problems involving apple cultivar improvement. The transfer of disease resistance and other traits via protoplast fusion may be of value once the technical problems of protoplast isolation and regeneration are solved. Protoplasts could be successfully isolated and cultured from callus and suspension cultures of *Malus domestica* cv. *Jonathan*. Formation of embryo-like structures was induced from the protoplast-derived callus on media supplemented with IAA and BA. These structures formed roots but plants failed to develop. Protoplasts could be isolated from leaves but not from stems or petioles. Protoplasts from

the fruit paranchyma of "Golden Delicious" was used in characterizing ethylene production. Protoplasts of cotyledon origin were used in the study of the role and localization of sorbitol dehydrogenase (45).

Cultures of generative plant parts

Anther culture

Anther culture techniques have been used to study the process of microspore development and to produce haploid callus or plantlets or both (75). By obtaining haploid plants and then doubling the chromosome number, it is relatively easy to produce pure lines for detailed genetic investigations, as well as for the heterosis breeding of apple. As cited by Milewska, the first studies on the *in vitro* culturing of apple anthers with the aim of obtaining haploid forms were carried out in Japan (57).

Anthers of *Malus domestica* cv. *Jonathan* were cultured on a modified MS medium (1962). After few weeks of culturing globular 32 and 64 celled embryoids were obtained from the uninucleate microspores. Globular embroids obtained from pollen grains were raised to green torpedo embryos, attaining a length of 3 mm after 10 weeks culture. During the culturing of anthers in different stages of development (tetrade, uninuclear microspora, two-nuclear microspora) it was stated that callus tissue developed from the walls of anthers, in the microspora stage. This green tissue, sometimes colored by anthocyanine, had a very dense consistency. The Japanese researchers also obtained roots from callus (57).

Fruit tissue culture

The fruit tissue from a wide variety of species has been successfully cultured in vitro. In addition to the most obvious advantages of minimizing undefined variables, fruit tissue cultures also provide experimental plant material on a continuous rather than seasonal basis. This method is of interest also as a model system in the study of fruit cell surface relationships. Also cell separation and tissue dissociation in situ are involved in apple texture changes with reduced storage life and market quality. The first successful *in vitro* culture of apple-fruit tissue was carried out by Letham (53). Explants of apple (*Malus domestica* Borkh.) fruit cortex were used to establish tissue cultures of "Lodi Golden Delicious" and "York Imperial" harvested at various stages of maturity. Cell division and initial callus formation occurred in all cases and was not affected by fruit age. Visible calli appeared 8 to 10 days after the explant was transferred to the culture medium (81).

Morphogenesis in embryonic tissue cultures

Adventious roots, shoots, and embryos were formed in apple, *Malus pumila* cvs Phiriki and Golden Delicious embryonic tissues cultured under light and at 25 °C on a modified MS medium in the presence of various growth regulators or combinations of them. Of the different parts of the embryo tissue, the petiole of the cotyledon was most responsive in morphogenesis. The initiation of shoots and roots was localized at the petiole of the cotyledon. Adventious shoots, when transferred to the same medium containing 2 mg/l IAA, rooted and formed plantlets (66). In 1977 Levi and Maróti (54) reported that green callus tissues of apple (Malus domestica cv. Jonathan) had been obtained from the cotyledons, the root and shoot meristems, the young leaves and stems. These cultures can be used in the study of embryogenesis as well as to investigate the effects of different materials (plant growth regulators, mutagenes, herbicides in the apple tissue culture (54).

Current research on micropropagation of Malus sp. and problems

Most of the recent research on apple has concentrated on optimizing micropropagation of scion cultivars and rootstocks and on the field evaluation of tissue cultured plants. Some of the main considerations are as follows:

— *Medium.* The culture medium must be suitable both in its chemical composition and physical qualities. Various basic-media have been developed, some of them are listed by Broome and Zimmermann (6). As a rule the Murashige–Skoog (1962) medium has been widely used with modifications according to varieties (77). The rooting of in vitro plantlets can be achieved either by in vitro or in vivo techniques (73). Certain varieties e.g. "Granny Smith" prefer a liquid medium, whereas other species can be rooted more easily on solid media. According to certain authors rooting can take place also in perlite (69).

The pH of a nutrient medium is also a critical factor. In general its value ranges between 5.5–6.2 (77).

— *Use of antibiotics.* Contamination will always be a major problem in stage-I cultures if explants are obtained from trees in the field. Therefore, the primary aim in micropropagation is to obtain or produce pathogen-free stock plants. With this in mind, antibiotics have been incorporated into culture media to rid explants of contaminations. However, such studies indicated that the antibiotics were bacteriostatic and not bacteriocidal (24). The most troublesome problem is caused by endogenous bacterial contaminations so that in most cases the large scale bacterial contamination is not a consequence of inappropriate techniques (15). Certain researchers hold the opinion that by using the "classical antibiotics" it is not possible to rid the explants of those bacteria (2). Recently, the new products "Alcide" and "Incyte" have been reported to have bacteriocidal effects (24). An alternative to using antibiotics is to en-

Table 1

Survey of the in vitro propagation methods of apple

Variety of scion/rootstock	Age	Explant	Mode of propagation	Reference
Malus sylvestris	S + A	ST	AXB + C	(1)
"Cox orange pippin" M. 26, M. 27, MM 106	A	ST	AXB	(5)
EMLA 25 (Rootstock)	A	ST + AXB + C	AXB	(9)
Golden and Starking Delicious + 4 of their mutants (Yellow Spur, Auvil Spur, Red Spur, Well Spur).	A		AXB	(8)
Northern Spy	A + S	AXB	AXB + AdvB	(18)
M. domestica Borkh. cv. Jonagold	A	MT	AXB	(16)
Ornamental INRA Malus X PERPETU EVERESTE	A	ST	AXB	(17)
Golden Delicious	S	mhn	embr.	(19)
Golden Delicious, Yellow Spur, stark Spur Golden	A	ST	AXB	(21)
M. domestica. cv. Akero	S	SS	C	(20)
M. 9 (Rootstock)	A	ST	AXB	(29, 30)
Northern Spy	A	ST	AXB	(35)
M. 9, M. 25, M. 26, M. 27.	—	SI + LD	C	(34)
M. 26 (Rootstock)	A	ST	AXB	(39)
Malling Kent, M. Suntun, James Grieve, Coxs Orange Pippin, and Golden Delicious	A	ST	AXB	(40)
M. 25	—	R	C	(42)
Red Spur and Gold Spur	A	ST	AXB	(43)
Ougnoe, Chernomorskoe letneey	A	ST	AXB	(44)
Red Delicious	S	Cs	C	(46)
M. domestica Borkh. McIntosh	S + A	ST	AXB	(49)
Mac Spur and Delicious (Haroldred) M. 27, M. 9, M. 26, MM. 111, Macspur.	A	ST	AXB	(51)
M. Domestica Borkh cv. McIntosh, (Summerland Red McIntosh, Macspur and McIntosh Wijcik.	A	ST	AXB	(50)
Golden Delicious	—	ls + cs + h	C	(55)
MM. 104, M. 26, M. 27, and Starkspur the scion cultivar	A	ST	AXB	(60)
M. 4 (M. Pumila Mill) rootstock	A	ST	AXB	(61)
Ottawa 3 apple rootstock	A	ST	AXB	(62)
M. Pumila cvs. Phiriki and Golden Delicious	Seed	ET	C	(66)
(Malus Sp.) MM 104, MM. 106, MM. 109	A	ST	AXB	(28)
Granny Smith	A	AXB	AXB	(69)
Jonathan and Delicious	A	ST	AXB	(70)
Crabapple (M. spp.) cultivars	A	ST	AXB	(71)
M. domestica Borkh. cv. Antonovka	Seed	EA + Cs	C	(68)
Antonovka 313 (M. Pumila Mill) (rootstock)	A	—	AXB	(76)

Variety of scion/rootstock	Age	Explant	Mode of propagation	Reference
M. Sylvestris Mill cv. McIntosh	S	MB	AXB	(80)
M. domestica Borkh. Lodi, Golden Delicious, York Imperial.	—	FC	C	(81)
A2 (rootstock)	A + S	AXB	AXB	(84)
M. 7 (rootstock)	A	ST	AXB	(87)
Akero, McIntosh, McIntosh Wijcik, Gravenstein and M. 26	A	ls	AdB + C	(86)
M. domestica Borkh. cvs. Empire McIntosh, Delicious, Triple red	A	ST	AXB	(89)
Delicious and Vermont Spur Delicious 17 apple (M. Sylvestris Mill) cultivars	A	ST	AXB	(91)
(M. domestica Borkh), Delicious and its strains	A	ST	AXB	(92)
2^7 apple cultivars (see the original source)	A	ST	AXB	(93,94)

Abbreviations:
A = adult plant
AXB = axillary branching
C = shoot differentiation from callus:
AdvB = adventitious bud formation:
cs = cotyledone segment
EA = embryonic axis
ET = embryonic tissue
ls = leaf segment
FC = Fruit cortex
H= hypocotyl

LD = leaf disc
MB = meristems from buds
mhn = micropylar halves of the nucellus
R = roots
S = seedling
SI = stem internodes
SS = stem segment
ST = shoot tip

courage the rapid growth of shoots with the objective of producing bacteria-free shoot tips (93). Conditions favouring rapid shoot elongation, (e.g., a low concentration of cytokinin in the medium and dark incubation) may promote the formation of suitable shoot tips (5 mm or less in length) for excision and further culture (2).

— *Root induction by phenolics.* Many of the recent studies on in vitro propagation of apple concentrated on the use phenolic rooting cofactors to improve rooting. Of the many phenolics studied, the most controversial is the effectiveness of Phloroglucinol (PG). PG has stimulated rooting of some apple rootstocks and cultivars while having no effect on others. PG has also been shown to reduce the period for IAA- and IBA-induced root initiation. The

effect of PG was first demonstrated on shoot proliferation by Jones (38) for "M.7" and "M.26" rootstocks. Others found no advantage (28) whereas others reported on the growth inhibiting effect of PG (1). According to Zimmermann and Broome (90) the phloroglucinol has no consistent effect on the growth of apples. It is, however, most probable that it increases the formation of adventitious roots (30, 39).

According to Zimmermann the various responses could be due to the following factors:

(a) environmental conditions at the time of explant is taken
(b) physiological state of the explant and/or
(c) the number of subcultures prior to rooting.

The relative effectiveness of PG compared with other phenolics requires further investigation. Although it has been tested at several concentrations, other phenolics have been tested at only very high concentrations. Phenolic compounds and their glycosides are widespread in plants. They have uncertain metabolic roles, although they are regarded as metabolic by-products conferring resistance to pathogens. Phloridzin, the 2-glucoside of phloretic acid, e.g., is unique to species of Malus and is the major phenolic compound of commercial apple trees (*Malus pumila* Mill), representing 3–7% of the dry weight of leaves as well as occurring in bark and roots. It is not involved in resistance to apple scab as formerly supposed, but affects the metabolism of many organisms. One of the degradation products of phloridzin, phloro-glucinol, acts synergistically with auxin to promote growth. There have been indications that phloridzin and PG, promote both the expansion of apple leaves and the rooting of apple shoots. Phloridzin and PG also promoted internode expansion and increased the number of shoots per tip to between three and six of apple rootstocks "M.7", "M.26" cultivated in vitro (24, 30, 38, 90, 92).

— ***Browning of medium.*** A serious problem generally found in culturing adult tissues, is the oxidation of phenolic substances leaching out from the cut surface of the explant which turns the medium dark brown and is toxic to the tissues. Quick transfers of explants to a fresh medium two to three times, at an interval of a few-days, may in some casees alleviate the problem. During this period the cut end of the explant becomes seals over and the leaching stops. The initial culture in a liquid medium probably helps to get rid of the phenolic compounds and other growth inhibitors. Should the browning persist at each subculture the addition of antioxidants, such as cysteine-HCL (100 mg/l) ascorbic acid (50–100 mg/l) or citric acid (150 mg/l), to the culture medium is recommended. Polyvinylpyrrolidin (polycar AT or PVP), which can absorb phenolic compounds, has also been used to save tissues from the toxic effects of the oxidized phenols. Keeping the cultures initially in the dark is also reported to be beneficial (i.e., ligth promotes the oxidation of phenolics) (3).

— *The application of thidiazuron (n-phenyl-N1-1,2,3-thiadiazol-5-ylurea.)* A compound shown to have a high degree of cytokinin activity, has been shown to stimulate apple shoot proliferation at concentrations considerably lower than BA. Shoot proliferation was shown to continue after explants were transferred to a cytokinin-free medium on which shoots could be rooted after 1–2 subcultures (24)

— *Agar.* Both concentration and the brand of agar definitely influence growth and multiplication as well as shoot proliferation and vitrification in apples. Because vitrification can impede the successful transfer of micropropagated apple plants to in vivo conditions, it is notable that vitrification can be eliminated on certain combinations of agar and the gelling agent "Gelrite", according to recent studies. It is evident from all these studies that a gelling agent can influence the anatomy and physiology reaction of cultured shoots. Consequently it is recommended that the brand of agar or gelling agent be positively identified in any research publication (24, 72).

— *Environmental factors.* The main effect of light is the resulation of certain morphogenetic processes (1000–5000 lux), whereas root formation favoured the effect of dark-treatment. A diurnal light regime of 16 h day and 8 h night has been found satisfactory for shoot development (3).

The effect of dark and temperature during growth/development, as well as root initiation has also been studied. The optimum duration of darkness, at the beginning of the rooting stage was shown to range from 3 to 9 days. James (35) reported that temperatures from 22 to 29 °C during root initiation did not influence rooting, but his experiments were carried out under light.

— *Temperature.* Cultures are usually maintained at a constant temperature of 25 °C (3). At lower temperatures shoots first accumulated anthocyanins then became clorotic (3, 24, 92).

— *Seasonal effects.* It is fairly well documented that the culturing of fruit crops in vitro can be influenced by the time of the year at which the explant is taken. James and Hutchinson reported in 1984 (35) that the optimum time to establishing apple shoot in vitro, with minimum browning and contamination, is in the spring (24, 35).

— *Field performance.* An important aspect which is often overlooked in the descriptions of *in vitro* culture methodologies is the establishment of plantlets for planting out. Essential to any practical system of in vitro culture is not only a high multiplication rate of plantlet production from the explant, but also a high rate of survival during acclimatization and in the field (74). Studies of the field performance of tissue cultured apple cultivars indicate that micro-cuttings come to flower within 2 to 2.5 years demonstrating that rooting ability is not associated with reversion to the juvenile form. When over several years tissue cultured apples were compared to budded trees it was established that the former were more vigorous and as productive as those on clonal

rootstocks and possibly more stress tolerant (83). It was also observed that vigour and cropping efficiency was cultivar specific. Some cultivars on their own roots were less vigorous and fruited much less than trees on specific rootstocks. Some authors, however, indicate that these micropropagated trees were a year younger than the scion part of the composite tree (24, 79).

Conclusions

A considerable number of apple rootstocks (5, 9, 11, 12, 28, 29, 32, 39, 60, 61, 84, 87) and scion cultivars (1, 8, 21, 41, 43, 62, 89, 93) are being propagated in vitro. This trend is likely to continue in the future and will most likely be aimed at producing:

— virus-free stock plants
— new cultivars
— elite genotypes of difficult-to-propagate plants
— large quantities of rootstocks.

The propagation of scion cultivars sems to remain a persisting issue. A major problem is also cost. Labor costs can be reduced however, if procedures are shortened or simplified. Thus, Simmonds (73) shortened the procedure for "M.26" apple rootstocks by substituting rooting under mist for in vitro rooting. The rootstocks were dipped in commercial rooting powder and established in a peat: sand (1 : 1) substrate within a mist frame. With this procedure, a plant establishment above 80% was obtained. Fiorini and Leva (21) used even less expensive materials when they induced root initiation in a liquid basal medium. Zimmerman and Fordham (93) induced root-formation in an auxin-sucrose liquid medium and the root development took place in peat.

Another important problem in propagating scion cultivars in vitro is that differences exist among cultivars (51, 69, 93) as well as between the juvenile and adult forms of the same plants in their requirements towards culturing (84, 85). Therefore, general recomendations for procedures and media composition based on experiences gained by the culturing of juvenile shoots, or one certain cultivar, may be ill advised.

Field studies indicate that tissue cultured apples are exceptionally vigorous over a span of several years and are equally as productive as bud-grafted trees. Nevertheless, a micro-propagated rootstock may never be economically competitive with a conventionally propagated one unless the plant material is in short supply and there is a huge demand for it. A micro-propagated, self-rooted scion cultivar, however, stands a very good chance of being economically competitive with the high-priced bud grafted trees (24, 86). Consequently, the in vitro propagation techniques are likely to play an important role in the future production of apples.

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Acta Agronomica Hungarica, Vol. 40 (1–2), pp. 253–258 (1991)

Book Reviews

Poliprivredna Znastvena Smotra, Zagreb, 1989. Vol. 54. No. 3–4

This new volume of the Zagreb University publication again contains comprehensive information, intended for those familiar with agricultural research and production. The seven original articles and a scientific survey written in Croatian are provided with an English summary and table explanations.

Tests were carried out in the middle section of the Drava valley to determine the efficiency of the P and K fertilizer supply in a crop rotation of winter wheat, sugar beet and maize. The data related to the sugar beet were published. Compound fertilizer was applied in both cases with the monoammonium- and tricalcium-phosphate, and the potassium salt tested separately. The sugar beet yield was found to be approximately the same as the standard; however, the reducted costs represented a considerable advantage.

The influence of the duration of harvest on root production and sugar content was also tested in sown sugar beet and in stands propagated by cuttings. It was concluded that the prolonged harvest and the proper variety may play an important role in the profitability of sugar beet cultivation in North-Western Croatia.

Susuri et al. isolated some *Ascochyta pisi* with variable growth vigour and exposed then to light radiations of different type and duration. The most intensive sporulation occurred on potato-dextrose agar (PDA) at full light intensity and constant temperature.

Fasaic et al. carried out haematological and biochemical tests on finger-sized and two-year-old carps *(Cypriuns carpio L.)* grown under artificial and natural conditions, respectively. A significant difference ($P < 0.01$) was found between the test results.

Furman studied the major parameters of the agricultural machinery and workshops operating under improved conditions. The main factors included the specific resistance of the soils, the number of tractor units, the machine types and the structure of the cultivated crops.

According to the report of Milat, ample attention is paid in Jugoslavia to the marketing of wine and its connection with nutrition, as well as to tourism. The concept of modern wine marketing is based on technology improvement and good quality and, consequently, also on increased economic efficiency.

Ankica and Jakovlievski studied the optimal investment policy in agricultural production systems. Their objective was to make an account of the existing crop inventory and the machine stock with the use of various mathematical and economical models.

After the Chernobyl disaster the impact of radiation on plants and other living organisms was studied also in Jugoslavia. In the long run, the increased rate of mutations can induce harmful genetic effects in the living organisms. This aspect is detailed in the valuable study of Katarina Boroievic, a well-known geneticist at the Novi Sad University.

Z. Bedő

P. Biacs, Katalin Gruiz and T. Kremmer (Eds.) — *Biological Role of Plant Lipids.* 1989. Joint edition by Akadémiai Kiadó, Budapest, Hungary and Plenum Press, New York and London, ISBN 963 05 5375 9)

The book is a collection of the papers presented at the 8th International Symposium on the Biological Role of Plant Lipids, organized by P. Biacs and his colleagues in Budapest on July 25–28, 1988. The book also contains those papers which were read at the post-symposium round-table discussion on the thermal acclimation and temperature stress, organized by T. Farkas, I. Horváth and L. Vigh at the Biological Research Center of the Hungarian Academy of Sciences in Szeged, Hungary.

The book comprises seven chapters, the *first chapter* of which deals with the lipid metabolism, with 23 papers (166 pages). The *second chapter*, "Structural and Functional Organization of Lipids" contains 23 papers (106 pages). The topic of the *third chapter*, a collection of 11 papers (54 pages) is the "Biosynthesis and Function of Prenyllipids."

The 10 papers (53 pages) in *chapter 4* involve a large and new field as its title indicates: "Carrier Proteins, Genetics of Plant Lipids." These works deal mainly with various problems of the movements of the lipids from the standpoint of molecular biology.

Chapter 5, "Biocides, Interaction with Plant Lipids" (13 papers, 52 pages) provides interesting data on the action of different chemicals that inhibit the lipid metabolisms, and on the molecular mechanisms of their actions. These data are of interest not only as they pertain to agronomical applications but also as they offer a better understanding of the biosynthesis of plant lipids.

Chapter 6, "Biotechnology of Lipids, Nutritional Aspects" (11 papers, 53 pages) is more complex, containing papers on a variety of topics, e.g. plant lipids as renewable sources of industrial surfactants; noncaloric fat substitutes on the basis of sucrose-esters of carboxylic acids; minor components of vegetable oils, etc. Their common point is the importance of their practical applications.

The *last chapter*, "Development, Environment, Stress" (28 papers, 114 pages) present many recent data on the various stress-effects, such as the effects of thermal stress, water stress, osmotic stress etc.

The book concludes with an Author Index, a very useful Subject Index and an Index of Taxa.

Obviously, this International Symposium was an extraordinarily large one, involving many aspects of lipid biochemistry. Thus, its material holds interest not only for the specialists. Although not intended as a school text, the book is useful for graduate and postgraduate students in explaining current problems of lipid biochemistry, and the modern methods by which these can be solved. The items published here are not abstracts or summaries, since they contain figures and tables in the text, many of which are even as long as regular papers in journals. They all end with references which are valuable for readers who work in other fields of biochemistry, and who require easily available information about the problems and the methods of their investigations.

L. Boross

John Ryan and Abdallah Matar (Eds.): *Soil Test Calibration in West Asia and North Africa*, International Center for Agricultural Research in the Dry Areas, 1990, Aleppo, Syria

The volume contains the Proceedings of the Third Regional Workshop on the Soil Test Calibration Network for West Asia and North Africa held in Aman, Jordan, 3–9 September, 1988.

The Workshop was attended by participants from ten countries and international organizations from the region and aimed to present the state and programmes of the soil testing methods and of their practical application.

Network meetings have been regularly organized and the results of the past inves-

tigations, as well as the achievements in the practical application have been presented in the different papers. Altogether 21 papers are published in the volume. The topics cover the different approaches of soil testing methods and of their evaluation for practical purposes.

The *first paper* of F. E. Khasawneh entitled "An overview of soil testing" gives a general review on calibration methods and factors affecting the soil test calibration data, as well as on the task of the soil test calibration.

The *following papers* represent mainly the problems of soil testing methods for measuring the available plant nutrient contents and elaborating recommendations for up-to-date fertilizer application. Examples are selected from different Mediterranean, Near and Middle East countries as Morocco, Pakistan, Jordan, Tunisia, Yemen, Turkey, Syria, etc.

Both theoretical and practical studies are included among the Proceedings, and up-to-date methods of modelling and process-predication can also be found among the papers.

Additionally, a great number of papers studied the methods of practical application of different fertilizers, mainly phosphorus fertilizers in the practice of rainfed and irrigated agriculture. A great variety of different soils and crops are represented in the different studies and the critical examination of conventional soil and plant testing methods have also been investigated.

Besides the professional papers, the addresses and acknowledgements of the Workshop are also published and an executive summary and conclusions facilitate an evaluation of the programme and the results of the Workshop for the reader of the book.

The Proceedings summarize a given phase of a long-term project activity of the countries and institutions included into the calibration network group and clearly show both the problems and the results of the important questions in soil testing and advisory service of the area.

I. SZABOLCS

"*Beitrage zur Tropischen Landwirtschaft und Veterinärmedizin*", No. 2/1989 and No. 3/1989 of the Journal well illustrate how serious investigations are being carried out, and how important scientific results have been obtained by the researchers in the countries of the tropical area.

The authors point out that where large proportions of Fe and Mn are in a dissolved state in the soil, due to flooding, and earthen drainage systems are used, the oxidation of Fe and Mn may cause a continuous lodgement of Fe and Mn over the inner surfaces and at the joints of the pipes.

The studies emphasize that traditional technologies may even be employed for the sake of progress in economically backward tropical regions, considering that the application of top technologies is not always the only way of developement.

The authors analyse the efficiency of growing various crops in rotation, e.g., in the case of soybean and sugar-cane.

A paper tries to find a solution to one of the most urgent problems of Africa: how to stop starvation, through possible ways of development in the cereal production of Ethiopia.

A special value of the two numbers of the journal is that they analyse the development of agricultural production in connection with the economic management, the property relations, and the agricultural organization units being formed accordingly, e.g., state farms, co-operatives and private small producers too.

I myself think it important to amalgamate the advantages of large-scale farms — better possibilities of mechanization — with the proprietory view of small private producers, so as to increase the agricultural production.

Analyses done in the form of case studies are more favourable methods of disclosing and solving the problems of agriculture than either the nation-wide studies or the simple data analyses.

S. ZSARNÓCZAI

R. T. HANLIN: *Illustrated Genera of Ascomycetes* (with drawings by Hahn, C. G.). 1990. 1–263 pp., Figs. 100, APS Press, The American Phytopathological Society, 3340 Pilot Knob Road, St. Paul, Minnesota 55121, USA — ISBN 0—89054 107–8.

The book discusses 100 genera of *Ascomycetes*, 65 of which are pathogenic to plants.

The author by a new concept of systematization divides the *Ascomycetes* into 10 families, and characterizes the families mainly by the colour and shape of ascospores and the number of their septa.

Families: *Hyalosporae*, *Allantosporae*, *Phaeosporae* (the ascospore is unicellular, oval or cylindrical, colourless or brown), *Hyalodidymae*, *Phaeodidymae* (the ascospore is a colourless or brown didymospore), *Scolecosporae* (the ascospore is filiform, uni- or multicellular), *Hyalophragmiae*, *Phaeophragmiae* (the ascospore is multicellular, colourless or brown), *Hyalodictyae*, *Phaeodictyae* (the septum of the ascospore is length- and crosswise, the ascospore is colourless or brown).

The genera are treated in the following way: On one side the genus is described in words according to the following subjects:

— Morphological characterization
— Description of the anamorphous form
— Habitat
— Representative species (illustrated in detail as a characteristic species of the genus)
— Comments
— References

On the other side, on the full page, the morphological characters necessary for the identification of the genus are seen in drawings and partly in photos, supplemented with figure captions. Many figures even show the symptoms visible on the plant.

An acquaintance with the morphological characters of the species representing the genera makes it easier to identify other species of the respective genera.

At the beginning of the book a taxonomic key for the genera is given, while at the end of the book a detailed bibliography can be found.

The book is considered an important source for those teaching phytopathology and for researchers specially interested in the identification of fungi.

M. GLITS

Poljoprivredna Znanstvena Smotra, 1989. Vol. 54. 1–2, Zagreb (Agricultural Conspectus Scientificus)

The quarterly periodical published by the Agricultural Faculty of the Zagreb University contains this time seven original scientific papers. These papers primarily summarize the results of plant cultivation and — breeding researches in Croatian, and are completed with English abstracts and table captions.

The authors of the first paper — Butorac, Basic, Vajnberger and Mihalic — deal with an actual question, changes in the fertilization methods of wheat. In a winter wheat — sugar-beet — maize rotation experiment set up on hypogley soils of the Midstream Drava Valley they studied the effect of maintenance fertilization with phosphorus and potassium in a nine-year experiment series. The phosphorus and potassium doses were calculated for a full rotation cycle. According to the results, the maintenance fertilization with phosphorus and potassium did not increase the grain yield, but proved useful from the point of view of economic production.

After inbreeding over eight generations, Parlov et al. detected in the Beljski Zuban 34 line a monogenically transmitted factor of resistance to Northern leaf blight, a leaf disease caused by *Helmintosporum turcicum*. The test crossing results proved a high general and specific combining ability for the grain crop, leaf number and size. The Bc 14 line produced from Beljski Zuban showed a particularly good combining ability with the lines Iowa Stiff Stalk Synthetic and Lancaster.

Matotan studied the relation between row distance and certain agronomical characteristics in soybean. With a reduction of the row distance from 60 to 20 cm the weight of

bean decreased, the first fertile node was on a lower level, the plant height and pod number increased. Similarly the yield of bean and the protein content increased.

In the course of microcloning vine cultivars, Hartl and Jelaska studied the effect of cytokinin and benzyladenine. The propagated plants were of juvenile type. The authors call attention to the dangers of BA overdosing.

Studying *Rhizobium leguminosarum* strains, Jarak found them to be most resistant to penicillin and least so to gentamycin. The author examined the number of pea nodes, the weight and height of the inoculated plants and the nitrogenase activity.

The effects of various plant growth regulators were examined by Dubravec in rape. "Boronet 70 WP" and "Boronet 70 WG" reduced the length of internodes and stimulated branching off. The stalk became shorter and more compact, and resistance to lodging increased. The growth regulators applied had a beneficial effect on flower formation, seed development and uniform ripening. The quality of seed crop did not change.

The papers listed above supply readily applicable data useful not only for agricultural research but also for the practice of crop production, so the periodical may command interest among a wider circle of readers.

Z. BEDŐ

Poljoprivredna Znanstvena Smotra, 1988. Vol. 53. No. 3–4, Zagreb (Agricultural Conspectus Scientificus)

The Croatian agriculture with its European- and world level production has deserved the recognition of international professional circles. The prestige acquired is largely due to the traditional research background which is a firm basis of agricultural development not only in Croatia but all over Jugoslavia as well.

The journal "*Agricultural Conspectus Scientificus*" edited in Zagreb offers an excellent cross-section of the wide scientific work which through its results is of much greater importance than would be expected from the Croatian agriculture on the basis of the size of its area. The many-sidedness of the agriculture is partly due to the wide variation of the ecological conditions, such as the topographic climatic differences. The journal, written in Croatian and completed with English summary and table as well as with figure caption, describes the soil types of Croatia.

In the crop production an important role is played by maize. The inheritance of the saturated fatty acid composition of the grain crop is dealt with by Surlan-Momirovic, who points out that the palmitic acid- and stearic acid content are of monogenic inheritance, as is supported by the high value of heritability for both fatty acids.

The effect of nitrogen fertilization on soybeans sown to different plant numbers was studied by Varga et al. According to the results of examination, in symbiosis with *Bradyrhizobium japonicum*, the oil contents of soybeans were in negative correlation with all nitrogen doses applied, and the N content of the bean increased, while the yield remained unchanged in the different treatments.

In a summary of a valuable work, the results of an approximately 15-year experiment series is presented by Ivanek, who studied the production potential, quality and botanical composition of meadows as a function of the mechanical structure of the soil.

Two papers deal with plants parasites and pests. Ostrec isolated 22 nematode species from tobacco, which mostly belong to the genera *Meloidogyne* and *Partylenchus*. In the course of his investigations the author discovered two new *Meloidogyne* species. In the other paper the dynamics of potato leaf aphid populations is discussed on the basis of seven years of observation.

Among the scientific papers related to animal breeding, the one written by Mioc et al. on body weight increase under intensive conditions of goat breeding deserves special attention. Studying the insemination of Holstein-Frisian heifers Uremovic et al. found that, with an intensive feeding, the

heifers could be inseminated earlier, when their body weight reached 350 kg and they were 450 days old. Problems of chicken fattening are dealt with by two authors: Mujkic and Jeleca.

In the economic section Radinovic analyses the change in the production capacity of Dalmatian private farms. As in many parts of the world, the number of private farms shows a slow decrease, and the size of the cultivated area has also become smaller. It is a good thing, on the other hand, that the degree of mechanization increases.

Studying the changes in the food consumption habits in Jugoslavia, Kero points out that when the living standard was rising, until 1979, consumption increased of all kinds of food, particularly meat, egg and beer. Since the deterioration of the life conditions, milk-, oil- and beer consumption have further increased, while consumption of other foods has either declined, or stagnated; this especially applies to fruit and meat!

The *Agricultural Conspectus Scientificus* is safely recommended to those enged in agricultural research work as well as to innovative practicers of the profession interested in the results of research.

Z. BEDŐ

PRINTED IN HUNGARY
Akadémiai Kiadó és Nyomda Vállalat, Budapest

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Index: 26.001

Acta Agronomica Hungarica

VOLUME 40, NUMBERS 3–4, 1991

Akadémiai Kiadó, Budapest
ACTA AGRONOMICA HUNG. HU ISSN 0238–0161

ACTA AGRONOMICA

A QUARTERLY OF THE HUNGARIAN ACADEMY OF SCIENCES

Acta Agronomica publishes papers in English on agronomical subjects, mostly on basic research.

Acta Agronomica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ

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VOLUME 40

AKADÉMIAI KIADÓ, BUDAPEST
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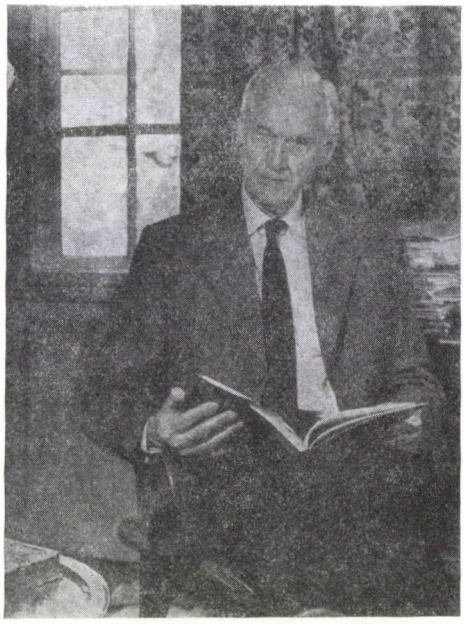
PROFESSOR DR. ARTHUR HORN AT EIGHTY



The Great Old Man of the Hungarian animal breeding science: ARTHUR HORN, distinguished professor and academician was born in 1911 in Cairo, Egypt. He graduated from the University of Technical Sciences in Budapest. In 1947 he became professor of animal husbandry at the University of Agricultural Sciences, Gödöllő. Since 1963 he has been an ordinary professor of animal production and applied animal genetics at the University of Veterinary Medicine at Budapest. He has been consultant, lecturer and guest-professor at a great number of universities in Europe, USA, India and Latin America, and the invited main speaker of a number of international and world-congresses, conferences and symposia, especially in the field of milk and beef production and genetics of domestic animals. He was also a consultant to the FAO for four years. For decades he was a leading personality of the European Association for Animal Production, and was also the vice president of the EAAP. He is an honorary member of the Belgian Academy of Medical Sciences, the German Academy of Agricultural Sciences (Berlin), the Polish Academy of Sciences, the British Cattle Breeders' Club, etc. He is an honorary Doctor of the University Halle-Wittenberg, Brno, Budapest (Univ. of Vet. Med.) and Gödöllő (Univ. of Agric. Sci.), and a full member of the Hungarian Academy of

Acta Agronomica Hungarica 40, 1991
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Sciences. He has been awarded with a number of national and international prizes, such as the Italian "Golden Egg", the State Prize of Hungary and the Golden Medal of the Hungarian Academy of Sciences. Besides being the author, co-author and editor of 13 books (some in several languages) and about 220 scientific publications, his scientific activity has been directed towards applied animal genetics, utilisation of the heterosis effect, and the integrated evaluation and efficiency of different populations and types, especially in milk and beef production.

The large number of students and members of the Horn's school congratulate their Master and wish him many happy years and good health, enjoying the results of his great work!

J. DOHY

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PROFESSOR DR. IMRE MÁTHÉ AT EIGHTY

DR. IMRE MÁTHÉ who has devoted all his life to the scientia amabilis celebrated his 80th birthday on 21st January 1991.

He completed his secondary school and university studies in Debrecen, his native town. Even in the lower grades of the secondary school, he was interested in botany, and his love of this subject brought him to the university where besides botany he studied geology and chemistry. The highly talented student soon attracted the attention of Professor Rezső Soó, Head of the Department of Botany. In the research work the Professor regarded the young demonstrator, at the time an assistant, as his colleague. In his doctoral dissertation, that he defended in 1933 "summa cum laude", Imre Máthé dealt with the flora and vegetation of the Ohat forest in Hortobágy.

From his early scientific work, the study of the alkali forests in the Kőrös region, the description of the flora in Trans-Tisza, and the flora-element analysis of the plants of Hungary deserve special mentioning. The latter work has been used up to now when placing the species of the Hungarian flora. In 1941, directing the Botanical Institute and the Botanical Garden, he held lectures in botany at the Debrecen Academy of Agriculture.

From 1942 he was brought into an ever closer connection with agricultur-

al botany; first at the Academy of Pallagpuszta, then from 1949 at the Budapest University of Agricultural Sciences he was Professor and Head of the Department of Botany. On two occasions he was Rector of the University.

In the framework of agrobotanical research he dealt with the dry cultivation of rice, and with the association aspects of various grass- and papilionaceous species. This scientific activity of his was acknowledged by the Hungarian Academy of Sciences, which in 1954 elected him a corresponding member. His research work was rewarded with the Kossuth prize in 1955.

Besides his duties as rector and his science policy activity in academic committees, he directed the educating- and research work of his department. The members of the department carried on examinations of the ecological and cenological conditions of various meadow associations of Hungary under his guidance.

In 1956 he was Rector of the University which was enough to transfer him to the Research Institute for Medical Plants. Characteristically of his love of the profession he found here the right field of research and opened a new scope of science in medicinal plant research. He studied the relationship between the ecological characteristics of the indigenous medicinal plants of Hungary and the production of the active agents and the biologically active substances.

He published many papers with his collaborators on the genera *Achillea*, *Matricaria*, *Artemisia*, *Vinca*, *Colchicum*, *Atropa*, *Solanum*, etc.

From 1964 he worked at the Botanical Research Institute of the Hungarian Academy of Sciences as scientific consultant. In addition to his investigations into medicinal plants he was participant or even manager of many ecological programmes. In the framework of the International Biological Programme (IBP) he and his collaborators carried on phenological and production biology examinations on the nature conservancy area at Újszentmargita. Owing to his wide international relations, he took part at the UNESCO session in Paris and Rome.

He was an active participant of International Botanical Conferences in Edinburgh, Seattle, Leningrad and Berlin. His scientific activity is shown by more than 300 Hungarian and foreign papers, and parts of books. As a recognition of his activity he became a regular member of the Hungarian Academy of Sciences in 1970. He is member of various scientific bodies of the Academy, of editorial boards of journals, and even today is leader of the editorial board of the series "Cultivated Plants of Hungary."

Besides his educational and research work, as well as scientific public activity, he has always been patient and attentive with his co-workers and students. Many of his Hungarian and foreign students can call him their master. Several of his former co-workers and students are today professors of various university departments.

The Hungarian botanists greet him with honour and love on the occasion of his 80th birthday, and wish him strength and health for his further work.

MARGIT KOVÁCS and A. KOLTAY

Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 263–272 (1991)

Soil science and agrochemistry

THE EFFECT OF SELECTED ORGANIC SUBSTANCES ON THE BEHAVIOUR OF WATER CONTAINED IN SOIL

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(Received: 2 April, 1990; accepted: 10 July, 1990)

This paper presents the results of investigations of water behaviour during its flow through the membranes of loess samples, modified with dodecylamine hydrochloride (DDAHCl) and oleic acid. The measurements of water filtration and sedimentation time, as well as the volume of water and sedimented soil, depending on coverage degree of the surface with the above modifiers, were made. The conclusion is that the above parameters depend strongly on the nature of the substances investigated and on the mechanism of soil surface adsorption. The substances for modification may be used successively to regulate of the rates of penetration and filtration of water, as well as evaporation of water from the soil.

Keywords: loess membranes, organic substances, water films, water penetration

Introduction

A modern agriculture practice utilizes many methods of improvement of such physical properties of soil medium as soil structure, water-air ratios, mechanical properties, salt content, etc. These methods include mechanical tillage, liming, hydration and dehydration, organic fertilization and drop rotation. Traditional methods for the improvement of soil medium physical properties are insufficient for the conditions of plant production intensification, because they require long-term use (Allison 1973). Modern agriculture lacks sufficient amounts of organic fertilizers to compensate for the difference in humus compounds and polysaccharides; and the stability of natural structure-forming substances contained in the soil is low because of the rapid mineralization of these compounds by microorganisms.

A recent attempt to increase the effectiveness of traditional methods, using the synthetic structure-forming substances, has been observed (De Boldt 1972). The use of the synthetic substances should guarantee the stability of formed soil structure, advantageous changes of physical, physicochemical properties of soils and profitable economic-production effects. Simultaneously, these substances should be characterized also by very slow decomposition by

high activity in the soil. The soil properties described above may be improved owing to the use of linear polymers as well as other chemical substances such as ion exchangers, surface active agents and different industrial waste materials (Allison 1952). The investigations of the use of synthetic substances for property improvement soil have shown that these substances improve the structure of soils (relaxed soil does not undergo encrustment, surface caking disappears) and their water capacity. This is because introduction of chemical substances into the soil induces complex changes in physical and physico-chemical properties of the solid-liquid system (Weyl and Ormsby 1960). The presence of hydrophobic or hydrophilic substances on the surface of soil particles influences their moisturability and thus determines their motions and retention in the soil. Depending on the type of preparation used, synthetic compounds cause the changes of intensity of water evaporation from the soil surface, whereas hydrophobic surface active agents cause the decrease of the rate of capillary rise due to the formation of a dry surface layer, which acts as a diffusion barrier.

The amount of water retained in the soil depends strongly on physico-chemical properties of the soil surface as well as its mechanical composition, profile, etc. (Low 1979; Low 1982). Water contained in the soil possesses differentiated mobility, depending on the degree of its bonding with the soil particles (Kowalik 1973). For this reason under different conditions many possible states of saturation of soil particles with water can exist (Staszczuk and Waksmundzki 1982, Chibowski and Staszczuk 1988). The regulation of water content in soil is one of the most important agricultural and drainage activities.

The presence of polyelectrolyte emulsion polymers or other substances adsorbed on the surface of soil particles can change either sorption properties of soils, or their sorption capacity (kinetics of sorption, its value and bonding energy) (Popiel and Zyla 1977, Lee Swartzen-Allen and Matijewic 1974). It has been assumed in general (Dechnik and Debicki 1977) that the introduction of natural and synthetic compounds that improve the soil properties facilitates the accessibility of nutrients to plants. Moreover, some of these compounds are also the source of the nutrients. Owing to the improvement of soil properties, better conditions of life appear for oxygen-requiring plants and a significant development of the bacteria then takes place. This fact was confirmed by the investigations of the CO_2 content in soils (Dechnik and Debicki 1977). Synthetic preparations, in turn, are characterized by a very high resistivity against the action of microorganisms (to 5 years) whereas the natural organic substances are decomposed during 2 months.

The above factors caused by the action of synthetic compounds in the soil have various effects on plant crops. These effects depend on the nature of soil and culture, as well as on the nature and concentration of the compounds used. Appropriate preparations may be used in the cultivation of sugar-beets

for the favourable concentration of their germination, whereas in the cultivation of potatoes the presence of such preparations accelerates their vegetation and thus permits an earlier harvest.

Considering all these factors, it can be concluded that further investigations on the use of synthetic compounds to improve soil properties are timely and advisable. In this connection, the paper describes the effects of soil sample modification with dodecylammonium hydrochloride and oleic acid on the parameters characterizing the behaviour of water during its flow through the membranes, using soil samples. With properly prepared loess samples the time of water filtration and sample sedimentation, as well as the volume of drained water and sample sedimented soil were measured.

Materials and methods

Materials

Measurements were carried out on loess samples, from the second outcrop in Elizówka, near Lublin, in the upper humus layer (depth 5—15 cm). Determined by the nitrogen thermal desorption method, the specific surface area of loess was equal to 24.8 m^2/g. Loess samples were dried before modification at 105 °C for 10 hours in order to remove the hygroscopic water.

Reagents

These reagents were used as the soil modifiers:

(a) Dodecylamine hydrochloride (DDAHCl) prepared from nonaqueous either solutions of appropriate aliphatic amines.
(b) Analytical grade oleic acid.
(c) Analytical grade methanol was used as the solvent solutions.

Two solutions were used in our experiments:

(a) Solution of dodecylamine hydrochloride in methanol containing 24.7×10^{-5} moles of amine in 1 ml which referred to 5 g of loess sample of specific surface area of 24.8 m^2/g corresponding to 1 statistical monolayer, assuming that the area occupied by 1 DDAHCl molecule is equal to 25 $Å^2$.
(b) Solution of oleic acid in methanol containing 0.25 ml of acid ($D = 0.8905$ g/cm^3) in 10 ml of solution which referred to 5 g of loess sample of specific surface area of 24.8 m^2/g corresponding to 1 statistical monolayer formed by oleic acid molecules, assuming that the area occupied by one molecule of acetic acid is equal to 25 $Å^2$.

Solutions of dodecylamine hydrochloride of concentrations corresponding to 0.25, 0.5, 0.75 and 1 of a statistical monolayer were used.

The 5 g samples of loess were placed in the glass bottles and flooded with the appropriate amount (depending on the required coverage degree) of DDAHCl alcoholic solution. The solutions within each bottle were filled up to 10 ml, in order to create the same conditions of spraying the solutions onto the loess surface. After careful mixing, methanol was evaporated at 50 °C, and then the samples were dried in a dryer at 50 °C for 3 hours. The dried samples were mixed every 30 minutes. After cooling, the bottles were closed and left for further investigations.

Oleic acid was sprayed in a similar way. Moreover, a standard sample (so-called "zero sample") flooded only with 10 ml of pure methanol was also prepared. The standard sample was prepared in the same way as the test samples.

Methods

Individual parameters were determined for three samples characterized by an equal coverage degree. Loess samples of the mass of 1.5 g were used for investigations. The measurements were made in a plexiglass column of 49 cm length and 7 mm diameter, equipped with a nylon wire, flannel and needle of 7 cm length and 1 mm diameter. Before each measurement, the walls of the column were carefully washed with water, and rubbed dry before the already prepared sample was introduced into the column. Then the column content was standardized for 15 seconds by means of a vibrator. The column content was flooded with 10 ml of distilled water from the burette in such a manner that at first water was dropped in order to prevent column aeration and then its remaining part was poured in. The measurement of filtration time started at the moment of first drop elution and ended with the last drop. The eluent was collected in the cylinder of 10 ml volume and after the elution, the volume of the eluted liquid was measured. As the next step, the samples investigated were quantitatively transferred from the column to the test-tube of 10 ml volume. The test-tube content was filled up with distilled water to 10 ml and then the test-tube was stopped. Next, the test-tube was inverted 10 times, positioned vertically in the test-tube stand and the sedimentation time was measured. The stop watch was turned on at the moment of positioning the test-tube and stopped immediately after clarification of the suspension. After the sedimentation was over, its volume was directly measured. Sedimentation time and volume were measured 5 times for each sample before the average values were calculated.

Results and discussion

Figure 1 presents the results of the measurements of filtration time obtained for loess samples covered to a different degree with dodecylamine hydrochloride (curve 1) and oleic acid (curve 2) in the amounts corresponding to 0.25, 0.5, 0.75 and 1, of the statistical monolayer sprayed on the surface. From the data presented in this figure it appears that the filtration time of 10 ml water portion through a loess membrane increases monotonically, with the increase of coverage degree of the surface with DDAHCl from 67 minutes for an unmodified sample, to 248 minutes for the sample covered with one monolayer of DDAHCl. For oleic acid, the curve illustrating the relationship between filtration time and coverage degree has a completely different course (Fig. 1, curve 2). The filtration time of water attained the maximum for the soil sample covered with 0.25 of the statistical monolayer of oleic acid (153 min) and then decreased to 28 minutes (for the sample covered with 1 statistical monolayer), i.e. to the value lower than this obtained for the "zero sample" (67 min). Thus, adsorption of oleic acid on the soil surface influences differently the behaviour of water in such a porous membrane. If the amount of adsorbed oleic acid exceeds 0.5 of the statistical monolayer, then penetration of water through the porous membrane becomes more facile. From the above measurements of filtration time it results that the mechanisms of penetration are different for both cases considered.

Simultaneously, the volume of water eluated from the column was also measured. As in the case of filtration time, the measurements of eluate volume were made for unmodified and modified samples. The average value of eluate for the unmodified sample was 9.15 ml. From Fig. 2 it appears that the increase

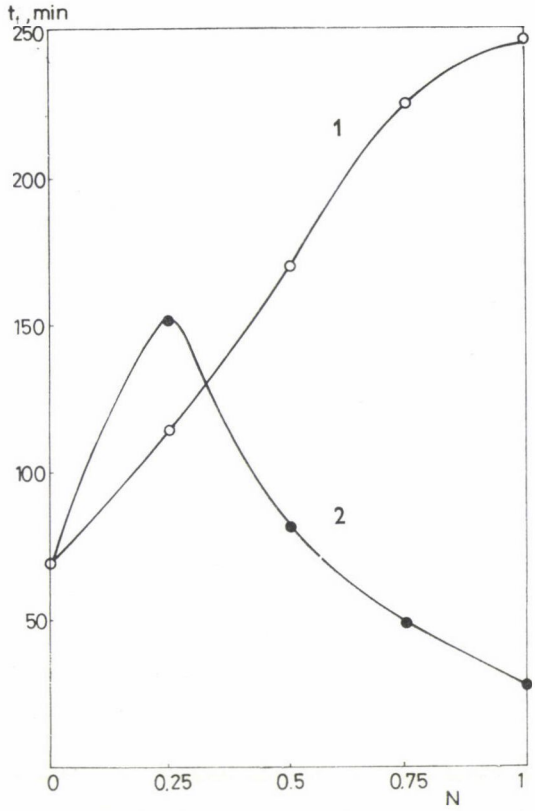

Fig. 1. The dependences of the filtration time t_f for the loess samples covered with DDAHCl (curve 1) and oleic acid (curve 2) vs. the amounts of the statistical monolayer N sprayed on the soil surface

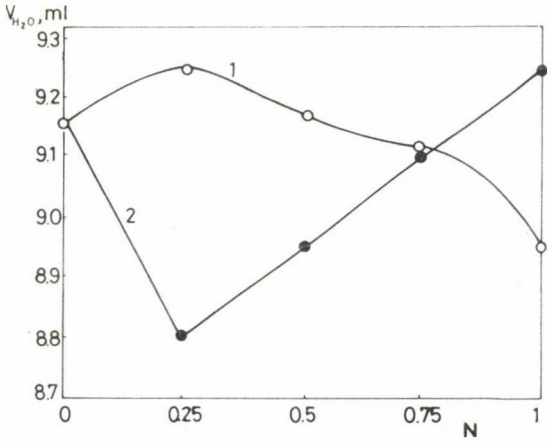

Fig. 2. The relationship of the volume of the water eluated V_{H_2O} for loess samples covered with DDAHCl (curve 1) and oleic acid (curve 2) and the amounts of the statistical monolayer N sprayed on the soil surface

of coverage degree of DDAHCl molecule surface (curve 1) caused a slight increase of the eluate volume, and during the further increase of the coverage degree, the eluate volume decreased to 8.95 ml. For the soil samples covered with oleic acid (Fig. 2, curve 2) the course of the above relationship is completely different. For the concentration corresponding to 0.25 of the statistical monolayer of oleic acid the eluate volume decreased to 8.8 ml and then increased a little more than that obtained for the pure (unmodified) sample. It should be noted that the presence of 0.25 statistical monolayer on the soil surface causes formation of a soil membrane which is the worst penetrated by the water (the longest filtration time — Fig. 1, curve 2, and the lowest eluate volume — Fig. 2, curve 2). On the other hand, the soil is the most permeable for the water when the soil surface is covered with 1 statistical monolayer of oleic acid.

After determining the filtration rate and volume of the water retained in the column, sedimentation time was measured. The investigated soil samples were quantitatively transferred to a calibrated test-tube and flooded with 10 ml of distilled water. After stoppering and inverting the test-tube 10 times, the measurements of sedimentation time were made. Five measurements were made for each sample and then the average values were calculated. The average value of sedimentation time for the unmodified sample was 91 seconds. The dependence of sedimentation time on the coverage degree of loess surface for the samples covered with DDAHCl is presented in Fig. 3 (curve 1) and for the samples covered with oleic acid in Fig. 3 (curve 2). Figure 3 (curve 1) shows a polyextreme course of the curve illustrating the changes of sedimentation time with the changes of coverage degree of loess surface with DDAHCl molecules. In a whole range of DDAHCl concentration, the values of sedimentation time for the samples covered with DDAHCl are higher than those of the unmodified sample. The curve possesses two maxima corresponding to 0.25 and 0.75 statistical monolayer, and a minimum corresponding to 0.5 statistical monolayer. A similar curve determined for oleic acid (Fig. 3, curve 2) has a slightly different course. In this case the maximum of the curve corresponds to 0.25 statistical monolayer of oleic acid, and then sedimentation time values decrease to the values significantly lower than those determined for the "pure sample" of soil. Just after clarification of the suspension the measurements, of sedimented soil sample volume were undertaken. For the unmodified sample this volume is 1.55 ml. Curve 1 in Figure 4 shows the experimental results obtained for the sample covered with DDAHCl, and curve 2 in this figure those results obtained for the samples covered with oleic acid.

As in the case of sedimentation time (Fig. 3, curve 1) the curve illustrating the changes of sedimentation volume for the samples covered with DDAHCl also shows a polyextreme course, whereas for the soil samples covered with oleic acid (Fig. 4, curve 2) the curve of the changes of sedimentation

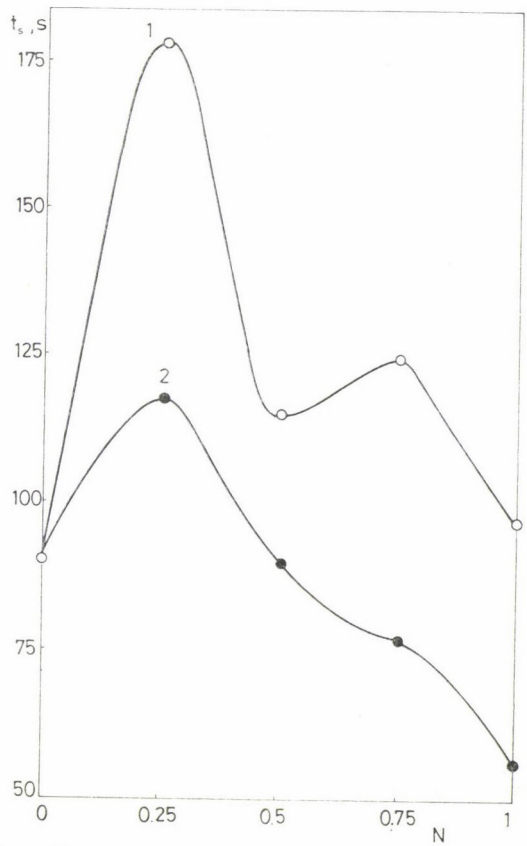

Fig. 3. Changes of the sedimentation time t_s of the loess samples covered with DDAHCl (curve 1) and oleic acid (curve 2) in relation to the amounts of the statistical monolayer N sprayed on the soil surface

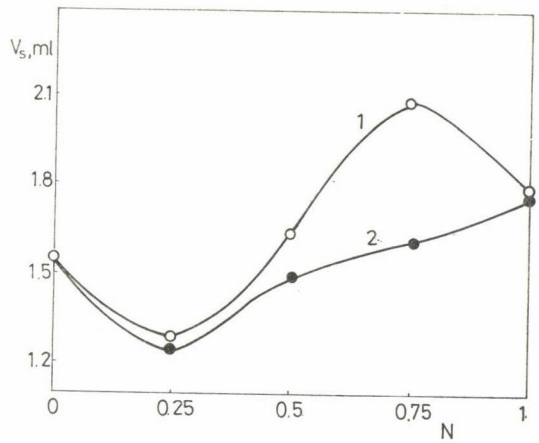

Fig. 4. Sedimentation volumes V_s of the loess samples covered with DDAHCl (curve 1) and oleic acid (curve 2) as a function of the statistical monolayer N sprayed on the soil surface

volume is a mirror image of the curve of the changes of sedimentation time (Fig. 3, curve 2). From Fig. 4 it appears that, for the coverage above, 0.5 statistical monolayer sedimentation volumes of the modified samples are, in general, higher than those obtained for "pure samples." This is probably due to the coagulation of the sample particles and in turn to adsorption of DDAHCl and oleic acid molecules. The interpretation of the changes of these parameters resulting from adsorption of DDAHCl and oleic acid on the surface of such a complex system as solid is very difficult. The differences in the effect of adsorption of DDAHCl and oleic acid on the behaviour of water in the porous diaphragms probably result from different types of adsorption of both substances and from different orientation of the molecules on the surface, as well as from the differences in mechanism of adsorption of these substances. The type of orientation of the molecules of modifying substances significantly influences the nature of water in soil, the depth of water penetration, its dislocation and assimilation by the plants. This influence manifests itself mainly in the formation of a vicinal water layer and its characteristics (thickness, structure, stability) and in the behaviour of the water contained in the capillaries and migrating among the soil particles (Kowalik 1973).

According to the recent conceptions (Staszczuk and Bilinski 1987a, 1987b) at relatively low coverages of the surface, i.e. about 0.25 statistical monolayer, DDAHCl molecules adsorb on the surface of hydrophilic solids through their own polar parts $(C_{12}H_{25}NH_3)^+Cl^-$ owing to chemical interactions with the polar active centers, whereas the hydrocarbon chain is oriented outside the sample surface. On such a "hydrocarbon brush," the molecules of reciprocal orientation can in turn adsorb, and owing to such adsorption, hydrophilic molecular clusters or macrocrystals (Fig. 5, a-1) can form. At low coverages oleic acid adsorbs chemically on exchangeable cations of soil surface with the simultaneous formation of a "hydrophobic brush" consisting of different oleates (soaps) (Fig. 5, b-2). The above hypothesis can be confirmed by the results of measuring filtration time and volume of water eluate passed through a soil membrane. At coverage degrees close to 0.25, the monolayer of DDAHCl "hydrophilic brush" of DDAHCl does not make the elution of water from the column practically difficult (for the covered samples the filtration time is only slightly greater than for the "pure" standard sample — Fig. 1, curve 1, and the eluate volume slightly lower — Fig. 2, curve 1). Hydrophobic "soap brush" in turn makes in the same coverage degree the eluation of water from the column difficult probably owing to formation in the membrane interior, the barrier repulsive for the water molecules (Fig. 1, curve 2 — the longest filtration time, Fig. 2, curve 2 — the lowest eluate volume). Thus, the significant increase of sedimentation time (which is equivalent to the time of clarification of the liquid column above the suspension) at the same coverage degree (0.25 monolayer) with DDAHCl (Fig. 3, curve 1) in comparison to

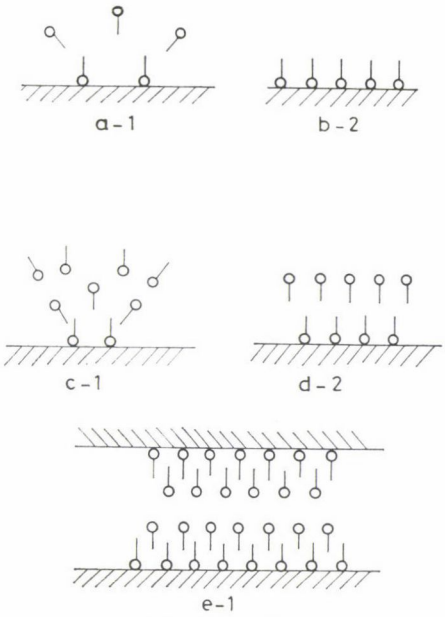

Fig. 5. The schematic structures of the DDAHCl (a-1, c-1, e-1) and oleic acid (b-2, d-2) layers adsorbed on loess surface

sedimentation time for the sample covered with oleic acid (Fig. 3, curve 2) reveals the peptization of colloidal particles contained in this sample.

The adsorption of other molecules of DDAHCl and oleic acid is probably of a physical nature with a reciprocal orientation of the molecules (Fig. 5, c-1, d-2). Owing to the formation of a "hydrophobic brush" (Fig. 5, c-1) the molecules of DDAHCl make significantly different the flow of water through the membrane (Fig. 1, curve 1 — increase of filtration time, Fig. 2, curve 1 — decrease of the eluate volume). In the case of oleic acid the presence of a "hydrophilic brush" (Fig. 5, d-2) causes the decrease of the filtration time (Fig. 1, curve 2) and eluate volume (Fig. 2, curve 2) and in this connection facilitates the flow of the water through the sample. Otherwise, the inflections of the curves (Fig. 3, curve 1, and Fig. 4, curve 1) are probably connected with a repeated reorientation of adsorption layer of DDAHCl on the soil surface. The sharp decrease of sedimentation time (equivalent to that of clarification of water column — Fig. 3, curve 2) reflects the coagulation of small particles leading to the formation of larger aggregates and to the disappearance of the surface charge (Fig. 5, e-1).

Summary

On the basis of the above investigations it can be stated that all parameters characterizing the behaviour of water in porous diaphragms of soil samples, modified with DDAHCl and oleic acid, depend on the nature of the substances, adsorption type and orientation of

the molecules on the surface. Chemisorption with hydrophilic (DDAHCl) and hydrophobic (oleic acid) orientation most significantly influences the above parameters. With the increase of the modifier concentration on the soil surface, the modifier molecules undergo reorientation leading to the formation of a hydrophilic or hydrophobic "brush". This is reflected in the form of the inflections on the curves illustrating the dependence of the change of individual parameters with the increase of the coverage degree.

When the amount of adsorbed water exceeds 0.5 of a statistical monolayer, then the flow of water through the soil sample significantly decreases, whereas the adsorption of above 0.75 of a statistical monolayer of oleic acid improves the penetration of water through the sample. The above conclusions can also have a practical application in agriculture because of the possibilities of regulation of the rate of water penetration and filtration through the soil bed, as well as the rate of its evaporation, using DDAHCl or oleic acid. They can be also helpful in the elaboration of methods preventing encrustment of soil, utilizing the above substances at appropriate concentrations.

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 273–279 (1991)

THE EFFICIENCY OF THREE COMMON EXTRACTION METHODS USED AS SOIL PHOSPHATE-TESTING FOR MAIZE GROWN IN SANDY SOILS

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(Received: 13 June, 1990; accepted: 1 November, 1990)

Surface samples (0–20 cm) of sandy soil selected from 4 sites varying in their P-status and $CaCO_3$ contents were treated with P-fertilizer as $Ca(H_2PO_4)_2 \cdot H_2O$ solution at rates of 0, 20, 40, 80 and 160 mg P kg^{-1} soil and incubated for 0, 3 and 6 months. Then soils were cropped in greenhouse with maize (*Zea mays* L.). The dry matter, P-content and P-uptake by maize tops were determined and correlated with the results of chemical soil tests (AL-, Olsen- and water-methods).

Quantities of P extracted by the three methods were significantly correlated with dry matter, P-content and P-uptake by maize tops when no P-fertilizer was added. In the mean of all rates of applied phosphate, Olsen- and water-extracted-P produced higher correlation coefficients only with P-content in maize tops. Concerning the relationships of P-content in soil and plant with incubation periods, Olsen and water extractable-P have relatively higher R-values than AL-extractable-P for all incubation periods. The soil samples were separated into two groups. The first group was characterized by no $CaCO_3$ content. The second group had a moderate amount of $CaCO_3$. The three methods produced a better prediction for P-availability for the first group than for the second group. The results of the estimation of available-P from chemical soil tests appear to be dependent on soil characteristics, incubation period and P-level applied. The water and Olsen-methods may be more advantageous in the prediction of available-P than the AL-method in the slightly alkaline soils.

Keywords: maize, *Zea mays*, P-nutrition, P-uptake, soil extraction methods

Introduction

Soil-testing procedures have been used with various success, to determine the phosphorus status of soils and to assist in preparing recommendations for phosphorus fertilizer use. There have been considerable efforts to determine the relationships between phosphate desorbed (i.e. soil test values obtained by various extractants and yields) and P-uptake to estimate the critical test values for optimum yield of crop for a particular set of soil.

Extraction procedures are regarded as being suitable if they successfully predict responses and are sufficiently simple to be used on a routine basis (Sorn et al. 1988). However, a large number of extractants can be grouped according to Sarkadi (1965) as follows: (a) mineral acids and their salts, (b) organic acids and their salts, (c) bases and salts hydrolizing basically, and finally, (d) water and neutral salts. It has been suggested that an ideal soil test extractant will dissolve a reproducible and consistent proportion of the labile-P, and

in addition it will reflect the extent and nature of reaction between the soils and any fertilizer phosphate that may be added (Thomas and Peaslee 1973).

Acid extractants such as ammonium lactate (AL) are superior for extracting the fixed part of fertilizer-P from the soil. In calcareous soils the available P-reserves are overestimated by the AL-method, but they are best shown by the Olsen-method (Balla 1978). It has also been recently determined that the quantities of phosphate extracted by $NaHCO_3$ were significantly correlated with the total phosphorus taken up by the crop (Tran Sen and Giroux 1987, Kuo et al. 1988, and Sorn et al. 1988). In contrast, Holford and Cullis (1985) found that the lactate test was superior and the $NaHCO_3$-test was inferior to other soil tests.

Adepoju et al. (1982) suggested that the estimation of available-P from soil analysis data might be dependent on soil characteristics (pH, soluble Ca, texture . . . , etc.). While Holford (1983) observed that the $NaHCO_3$ test was the most effective, and the Mehlich test was the least effective, on all soils studied regardless of sorptivity or pH of the soils.

Water-extraction of soil-P is one of many empirical soil-testing procedures used by several workers (Van der Paauw 1971, Ryden et al. 1977, Luscombe et al. 1979 and Sorn et al. 1988), who found that the water-extraction method is not greatly affected by soil type. In contrast, Smith and Gregg (1982) reported that water-extractable P was dependent on soil type. The objective of this study was to evaluate the efficiency of AL-, Olsen- and water-extraction as soil-testing procedures for P and to correlate these chemical soil tests with quantities of P removed by maize and with its dry matter yield as affected by soil characteristics, time and P-level applied.

Materials and methods

Four sandy soils varying in their $CaCO_3$ and phosphorus contents were collected from the surface horizon (0–20 cm) of the experimental farm of the Res. Inst. for Soil Sci. and Agric. Chem. at Őrbottyán in Hungary. Some chemical and physical properties of these soils are given in Table 1. The soils were air-dried, sieved (2 mm), and divided into 3 equal parts. The

Table 1

Some physical and chemical properties of the soil samples

Soil number	Previous fertilization	AL-P	Olsen-P	H_2O-P	AL-K	AL-Ca	pH	Humus	$CaCO_3$	Particle size distribution			
		mg/kg				%	(KCl)	%	%	Coarse sand %	Fine sand %	Silt %	Clay %
1	Ø	17	7	0	42	0.10	6.8	0.45	0.0	46.4	45.9	5.2	2.5
2	NPK	83	48	24	125	0.09	6.1	0.45	0.0	41.0	50.3	7.0	1.7
3	Ø	32	7	0	42	1.90	7.8	0.24	6.1	52.2	45.5	2.2	0.1
4	NPK	114	36	16	100	2.70	7.8	0.30	7.8	53.0	42.9	3.8	0.3

first part was mixed with 0, 20, 40, 80 and 160 mg P · kg^{-1} soil as $Ca(H_2PO_4)_2 \cdot H_2O$ as a solution plus 10 ml 1% orthocide solution as a disinfectant. This part was incubated for 6 months. After 3 months, the second part was treated with the above-mentioned phosphate and orthocide doses and incubated for 3 months. The third part was stored without treatment. Each treatment was done with 3 replicates. The first and second soil parts were incubated in open trays at about 25 °C. The soil moisture was raised to 60% of the field capacity of each soil and adjusted every 2 to 3 days gravimetrically with distilled water. Composite samples were taken before and after 3 and 6 months from each of the two incubated soil series for chemical analyses. At the end of the incubation period of the two above-mentioned series of the soil samples, the third part was treated with the earlier mentioned phosphate and orthocide quantities and composite samples were also taken from it. The incubated soils were air-dried and crushed to pass through a 2 mm sieve.

Each treatment received a basal dressing of N and K as solutions of $(NH_4)_2SO_4$ and KNO_3 at rates of 83.3 and 125 mg kg^{-1} soil, respectively. Then a uniform weight of each treatment (1.8 kg) was introduced into 2 liter plastic pots. Eight seeds of maize (*Zea mays* L., Pi-3901 hybrid) were sown and thinned to 5 plants/pot after germination. The pots were irrigated with distilled water to about 60% of the field capacity of each soil. The same doses of N and K were added 3 times during cultivation. The experiment design was a split–split-plot. The plants were harvested after 30 days from sowing, washed and oven dried at 45°C. Plant tissue samples were digested with concentrated H_2SO_4 and 30% H_2O_2 (Thamm 1973). P was measured colorimetrically in 4 ml of the digested samples, using vanadate-molybdate as described by Thamm et al. (1968). $CaCO_3$ % of soils was determined by calcimeter method (Page et al. 1982), O.M. was determined by wet-oxidation procedure (Jackson 1958), soil pH was determined in 1.0 n KCl (1:2.5) and the particle size distribution was determined by the pipette method (Black 1965). Extractable-P from soils was determined by (a) ammonium-lactate-acetate solution (AL) according to Egnér–Riehm–Domingo (1960). P was determined colorimetrically by ascorbic acid and tin II. chloride (Sarkadi et al. 1965), K and Ca were determined in the same extract by atomic absorption, (b) 0.5 M $NaHCO_3$, pH 8.5 according to Olsen et al. (1954) and (c) distilled water containing 0.01% polyacrylic amide according to the method of Van der Paauw and Sissingh (1969), and Schachtschabel (1966) by the modification of Sarkadi.

Results and discussion

Data for the P extracted by AL-, Olsen- and water-methods from soil samples taken before and after incubation for 3 and 6 months are presented in Table 2. The quantities of P extracted by AL extraction were consistently greater than those extracted by either the Olsen or the water extraction. The water-method extracted almost the lowest quantities of P. Generally the quantities of extracted-P by the three extraction techniques decreased with increasing the incubation time up to 6 months, except that AL-extracted-P remained nearly constant, and showed a small increase during the second period of incubation which indicates a desorption of P due to the strong extractive power of the AL solution. The decrease in extractable P after incubation in all soils was largest for the water extraction. The water-extractable P was decreased by about 37,34, 44 and 52% in soils 1, 2, 3 and 4, respectively, after 6 months of incubation.After the same period the AL-extractable P decreased only by 7 and 8.5% in soils 1 and 2 (non-calcareous soils), and by 5 and 0.6% in soils 3 and 4 (calcareous soils), resp. Otherwise, the Olsen extraction method was more suitable to show the reduction in extractable-P in calcareous soils (soils 3 and 4) than in non-calcareous soils (soils 1 and 2). Quantities of extracted-P were reduced by about 20, 10, 23.4 and 22.5% in soil 1, 2, 3 and 4, respectively. These results agree with those reported by Balla (1978): The same data also

Table 2

The mean averages of extractable-P by AL, Olsen and water solvents as affected by the incubation periods in mean of all P-levels

Incubation period (months)	Soil				$LSD_{5\%}$	Mean
	1	2	3	4		
			AL-P mg/kg soil			
0	57	130	79	165	2	108
3	51	117	76	162	3	101
6	53	119	75	164	3	103
$LSD_{5\%}$	3	3	3	3		2
Mean	54	122	77	163		104
			Olsen-P mg/kg soil			
0	35	78	47	80	2	60
3	29	72	39	67	2	52
6	28	70	36	62	3	49
$LSD_{5\%}$	2	2	3	2		1
Mean	31	73	41	70		54
			Water-P mg/kg soil			
0	27	53	36	50	1	41
3	18	39	25	32	1	28
6	17	35	20	24	2	24
$LSD_{5\%}$	2	2	2	2		1
Mean	20	42	27	35		31

reveal that the decrease in extracted-P by the Olsen- and water-methods was higher in the soils 2 and 4 which previously received fertilizer-P compared to the corresponding decrease in extracted-P in the unfertilized soils (soils 1 and 3). It can be concluded from the above results that water extraction is the most effective method to illustrate the effect of incubation time on quantities of P extracted from the four studied soils regardless of their P-status and $CaCO_3$ contents. This finding agrees with those of Van der Paauw (1971), Ryden et al. (1977) and Sorn et al. (1988).

Data in Table 3 show that the amounts of extractable-P obtained by the three extractants and for all the four studied soils were significantly increased with increasing the rate of phosphorus applied up to 160 mg/kg soil. It can be seen from the same table that the amounts of extracted-P from various soils were affected by the type of the extractant used, despite the marked differences existing between the investigated soils in their $CaCO_3$ and P-contents. The mean averages of extractable-P were 60, 23 and 9 mg/kg soil when no phosphate was added, and these values increased to 181, 106 and 69 mg/kg soil, respectively, at P-level of 160 mg/kg soil.

Table 3

The mean averages of extractable-P by AL, Olsen and water solvents as affected by P-levels in mean of all incubation periods

P-level mg/kg	Soil				Mean
	1	2	3	4	
		AL-P mg/kg soil			
0	16	80	32	112	60
20	28	92	46	127	73
40	39	110	60	145	89
80	65	133	89	180	117
160	119	194	159	253	181
Mean	54	122	77	163	104
		Olsen-P mg/kg soil			
0	6	47	6	33	23
20	13	57	16	45	33
40	19	64	27	58	42
80	37	85	53	83	65
160	77	115	101	129	106
Mean	31	73	41	70	54
		Water-P mg/kg soil			
0	0	22	0	13	9
20	5	29	9	21	16
40	13	37	18	27	24
80	28	50	35	43	39
160	57	74	73	72	69
Mean	20	42	27	35	31

To explain the relationships between dry matter, P-content and P-uptake by maize tops and the extractable-P by AL-, Olsen- and water-methods, the values of quadratic regression analysis (R) gave better results than the simple correlation coefficient (r) in this study.

Quadratic correlation coefficients (R) for the relationships between dry matter, P-content and P-uptake by maize plants and P-extraction by AL-, Olsen- and water-methods were calculated and presented in Table 4. Regression analysis of dry matter, P-content and P-uptake by maize, with the three methods of extraction, produced higher coefficients for the soils without applied-P (column 1) than for those with applied-P in the mean of all rates of application and for all incubation periods. The decrease in the R-value due to P supply was greater for dry matter than for P-content and P-uptake in all extraction methods used. R-values for the relationships between AL-P and dry matter P-content and P-uptake were always the lowest. The correlation coefficients (R) for the relationship between water-P and P-content in maize exhibited the smallest decrease due to P-application. Regression coefficients of the rela-

Table 4

Quadratic regression coefficients (R) for the relationship between dry matter, P-content and P-uptake by maize tops and P-extraction by AL-, Olsen- and water-methods

Soil N°		1–4	1–4	1–4	1–4	1–4	1–2	3–4
P-level (mg/kg soil)		0	0–160	0–160	0–160	0–160	0–160	0–160
Incubation period (month)		0–6	0–6	0	3	6	0–6	0–6
Number of samples (n)		36	180	60	60	60	90	90
y	*x*				*R*			
Dry matter weight	AL-P	0.91	0.53	0.59	0.57	0.49	0.73	0.57
	Olsen-P	0.95	0.63	0.66	0.68	0.61	0.73	0.62
	H_2O-P	0.95	0.60	0.67	0.66	0.64	0.69	0.57
P-content %	AL-P	0.80	0.65	0.64	0.68	0.66	0.92	0.67
	Olsen-P	0.87	0.80	0.79	0.81	0.80	0.92	0.80
	H_2O-P	0.89	0.84	0.82	0.82	0.87	0.92	0.83
P-uptake	AL-P	0.88	0.61	0.60	0.63	0.60	0.91	0.74
	Olsen-P	0.94	0.73	0.72	0.75	0.74	0.90	0.79
	H_2O-P	0.94	0.74	0.73	0.73	0.80	0.88	0.76

tionships of AL-, Olsen- and water-extractable P with dry matter, P-content and P-uptake by maize before and after incubation were compared. The data reveal that there are no measurable differences in the regression intensity with increasing incubation period, except the marked decresse for the relationship of dry matter with AL-P and Olsen-P values, and the increases for the relationship of P-content and P-uptake with water-P values. To compare regression coefficients of relationships of extractable-P with each plant parameter at all incubation periods and P-levels, the soils were separated into groups. The first group was characterized by the absence of $CaCO_3$, and tended to have a relatively low pH and low soluble Ca. The second group had a moderate amount of $CaCO_3$, tending to have relatively high pH and soluble Ca. The data show that the intensity of the regression coefficients for all three extraction methods with any of the plant parameters were higher in the first group than in the second group. The *R*-values for the second group were always lower with AL-P than with Olsen-P or water-P with all plant parameters, while in the first group, *R*-values were slightly higher for AL-P and Olsen-P than for water-P. However, the regression analysis of P-content in maize with extractable-P by all the extraction methods produced in the first group an equally high *R*-value (0.92). The data indicate that the three methods of P-extraction give a better prediction for P availability in the slightly acid sandy soils, when $CaCO_3$ was absent, than in the calcareous soils where Olsen- or water-methods were more effective to serve this purpose. Thus, the estimation of available-P

from chemical soil test appears to depend on soil characteristics, time of incubation and P-supply. These findings agree with most items of literature in the introduction.

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 281–294 (1991)

Plant physiology and biochemistry

EFFECT OF NPK-FERTILIZATION AND SPLIT APPLICATION OF N ON LODGING DUE TO WINDSTORM AND "HARVESTABLE" GRAIN YIELD OF MAIZE

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(Received: 13 April, 1990; accepted: 20 June, 1990)

In 14–15 year field experiments, the effect of NPK-fertilization and N applied at different times was studied on maize plants for their development at the 4–6 leaf stage, lodging due to windstorm, and grain yield. The maize was sown at a density of 60,000 plants/ha, and depth of 7 cm, on calcareous chernozem soil (20% sand, 40% loess-like powder, 20% silt, 20% clay), originally moderately supplied with N and K, and poorly supplied with P and Zn. At the time of flowering maize was lodged by a storm lasting 25 minutes with 80 to 90 km/h windblasts. The extent of grading for lodging was carried out on net plots prior to harvest.

On each plot, the extent of critical lodging, i.e. the percentage of plant population lodged to 70° or more, was calculated, too. Pioneer SC 3901 hybrid with excellent stalk firmness did not lodge on account of the windstorm.

Szegedi SC 444 hybrid showed the greatest lodging and critical lodging (>70°) in the absolute control ($N_0P_0K_0$) and in the K deficient ($N = 250$; $P_2O_5 = 150$; $K_2O = 0$ kg/ha) treatments. Lodging was relatively great on the P control plots ($N = 150$; $K_2O = 100$; $P_2O_5 = 0$ kg/ha) alike.

Increasing doses of P_2O_5 (50–200 kg/ha) and particularly of K_2O (100–200 kg/ha) reduced the degree of lodging and the rate of critical lodging, whereas higher (200–250 kg/ha) doses of N caused increased lodging. Dividing N into two halves (1/2 N in autumn, 1/2 N in spring, before sowing) also increased the degree of lodging, as compared with the treatment given the total N in autumn.

A better stage of early development of the plants resulted in a lower rate of lodging. Merely K-control plot ($N = 250$; $P_2O_5 = 150$; $K_2O = 0$ kg/ha) was an exception where lodging was much more vigorous as compared to the stage of development of the population.

Total grain yield of Szegedi SC 444 hybrid increased up to $N = 150$, $P_2O_5 = 50$ (100), $K_2O = 100$ kg/ha levels. Rates of 200–250 kg/ha N and 150–200 kg/ha P_2O_5 (above 200–250 mg/kg AL-P_2O_5 content) already decreased yield by 0.4 to 1.0 t/ha. Pioneer SC 3901 hybrid gave a similar response to NPK-overfertilization.

"Harvestable" grain yield by combine (defined as that of the population lodged less than 70°) of Szegedi SC 444 hybrid was the highest at the following levels: $N = 50$ (150), $P_2O_5 = 100$ (150), $K_2O = 100$ kg/ha. On plots given no potassium, the "harvestable" yield was only half of that received potassium in an adequate level. Distribution of N (1/2 in autumn, 1/2 in spring) resulted in a lower "harvestable" yield than when giving the total N in autumn.

Keywords: lodging, windstorm, maize, nitrogen, phosphorus, potassium, distribution of nitrogen

Introduction

No experimental data can be found in Hungarian papers concerning the connection between damages caused by spring and summer storms and the nutrient status of cultivated plants. However, as it could be observed in practice, storms in May or June can cause a marked lodging of cereal plants of high density formerly having been fertilized with large N-doses.

On the other hand, factors influencing the breakage of maize stalks before harvest are dealt with in a large number of papers. According to Campbell (1964) the stalk breakage in the case of hybrids of considerable productivity can be more common because:
(1) the heavier ears exert a larger mechanical loading on the stalks, and
(2) the translocation of assimilates from stalks and leaves into the grains is more intensive before ripening than in the case of open pollinated cultivars. Rosic et al. (1979) describe the loading of the maize stalks in a mechanical model. According to their investigations, stalk breakage occurs mostly at the 5th internod under the cob. Arnold et al. (1974) reported that K-fertilization induced later senescence of maize stalks when grown on a soil originally poor in K. They also found that K-increased the crushing strength, and the rind thickness. Kálmán et al. (1974) found that the determination of crushing strength in the above-ground 2nd internod section represents a suitable index when improving new maize hybrids with the aim to obtain a variety with better stalk stability. Hybrids that have the ability to absorb a greater amount of nutrients have stalks that remain green until the grain is harvested, and are thus much more resistent to lodging than those having senescent stalks at ripening (Josephson 1962).

At the same time, breakage of maize stalks may be a consequence of infections which can vary according to the plants' nutrient supply. Otto–Everett (1956) reports that stalk-rot of maize caused by *Gibberella sp.* increased together with rising N-doses, and decreased when K-fertilizer was applied. When using high doses of P-fertilizer, Kádár and Zilahy (1977) observed a considerable stalk breakage in a maize which was promoted by *Fusaria* infection (and appeared on the stalks). The extent of stalk breakage was partly counterbalanced by increasing the K-supply.

Liebhardt and Murdock (1965) observed two types of lodging in K-deficient plants: root lodging and stalk breakage. The root lodging was due to a restricted brace root system and, subsequently, to the breakdown of parenchymal cells in the brace roots. Stalk breakage resulted from disintegration of the parenchyma in the lower portion of the stalk. When K was given to these plants, a strong root-system and stalk developed.

Fisher–Smith (1960) studied the effect of NPK-fertilization on yield and on stalk breakage of maize. As an effect of N-fertilization, the number

of broken stalks increased by 20% on the K_0-plots, at a constant P-level, while K-fertilization diminished the quantity of broken stalks from 60–70% to 20–30% at the same NP level.

When increasing the plant population density of maize, lodging can be more definite. At high population density (Norberg et al. 1988; Gaska and Oplinger 1988) the decrease of maize lodging was observed when "Ethephon" (a stalk strengthener) was used, but in some cases grain yield decreased.

This paper includes the data of 3, 14 or 15 years long-term fertilizer field experiments concerning the lodging of maize plants caused by a windstorm. On July 20, 1983, winds lasting 25 minutes and having a speed of 80–90 km/h, accompanied by a precipitation of 10 mm (without ice) damaged the maize then in flowering stage during experiments at Nagyhörcsök. The extent of lodging on the plots differed according to nutrient supply. This phenomenon remained unchanged until harvest time. Precipitation amounted to 23 mm in April 1983, 105 mm in May and 10 mm in June, following a drought of 4 weeks, until 20th July. This period ended with the storm mentioned above.

In this paper, data are given about the connection between the nutrient supply of the soil and the grain yield of maize.

Materials and methods

The Experimental Station of the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences at Nagyhörcsök is situated in the area "Mezőföld". This area is relatively poor in precipitation (590 mm/year), and the fluctuation of temperature is rather large, so its climate is similar to that of the Great Hungarian Plain. The soil of the experimental station represents a variant of calcareous chernozem soils with a humus layer of medium thickness (50–75 cm) (Szűcs 1965). The work in the experiments discussed in this paper was started in 1968 (Experiments A-18 and B-18), and in 1969 (Exp. A-19 and B-19). The ploughed layer has on the average a $CaCO_3$-content of 5%, and a humus content of 2.5–3.0%. The AL-P_2O_5- and the AL-K_2O-contents, determined by using 0.1 mol/L NH_4 lactate +0.1 mol/L acetic acid, pH = 3.7 (Egner–Riehm–Domingo 1960) were 60 and 160 mg/kg soil in the experiments A-18 and B-18, and 100 and 190 mg/kg soil in the experiments A-19 and B-19, resp., at the beginning. According to the official methods in Hungary, and the limit values determined by these methods, the soil was very well supplied with Mn, satisfactorily with Mg and Cu, moderately with N and K, and poorly with P and Zn (MÉM NAK 1979, Csathó et al. 1989).

The design (randomized block) of the experiment, the applied treatments and the sequence of the different plant rotations in the first 3 cycles were described by Sarkadi et al. (1984). From the 2nd cycle on, the crop rotations were winter wheat-maize–maize-peas (for experiments A), and winter wheat-maize–maize-winter wheat (for experiments B).

Yearly applied NPK-fertilizer quantities in the experiments, having 20 treatments and 4 replications from the 2nd cycle on, summarized in Table 1. NPK-fertilizers were applied as Ca-ammonium-nitrate, potassium chloride and simple superphosphate. In 2 of the 4 replications the total amount of N-fertilizer was given in autumn, while in the other 2 replications this amount was evenly divided, and the first part was given in autumn, the second part was given early in the spring.

Deep ploughing (26–28 cm) in autumn and harrowing in spring were followed by seedbed preparation before sowing. In the experiment A-1815, maize hybrid Pioneer SC 3901, and in the experiments B-1815 and AB-1914, maize hybrid "Szegedi SC 444" were sown.

Table 1

Doses of fertilizers (in active agents)

Nutrient level	N	P_2O_5	K_2O	N	P_2O_5	K_2O
	(1. cycle)			(2–4. cycles)		
	kg/ha					
0	0	0	0	0	0	0
1	40	40	80	50	50	100
2	80	80	160	100	100	200
3	120	120	—	150	150	—
4	160	160	—	200	200	—
5	200	—	—	250	—	—

Sowing took place on the April 24, 1983. The number of seeds sown per hectare was 60,000, planted 7 cm deep, using a Rumanian sowing-machine of the type SPC-6. In all experiments, weed control was done by Afalon (2.5 kg/ha) + Maloran (3 kg/ha) before sowing, and Niptan before germination. In the experiment A-1815 the maize harvest took place on Oct. 27, in the experiment B-1815 on Sept. 26–27, and in the experiments A–B-1914 on Oct. 20 and 21, 1983. In each experiment the harvest was done by hand. In the end, stalks were removed from the experimental fields. We also estimated the grain yield which could be harvested by combine. In this case we assumed that a combine cannot pick up plants lodged more than 70°. Thus, "harvestable" yield means the grain yield of the plot minus the fraction which was lodged more than 70°.

In the experiment B-18 in the autumn of 1980, and in the experiments A–B 19 in the autumn of 1981, soil samples were drawn from the 0–20 cm layer at 20 separate points on each plot.

To determine the AL-P- and AL-K-contents of the soil, composite samples were prepared from the single samples. The analyses of the soil were made by the Station for Plant Protection and Agrochemistry at Tanakajd, on behalf of the Coordination Centre of the National Fertilization Experiment Network of the Institute for Agrochemistry and Soil Science of the Agricultural University at Keszthely.

On June 6, 1983 at the stage of 4–6 leaves, the status of plant development was evaluated: number 1 indicating the least developed, number 5 the best developed, plant stand.

As the maize hybrid "Pioneer hybrid 3901/380/SC" has outstanding stalk-stability, only a lodging of minimum extent was seen after the storm in the experiment A-1815, and for this reason no grading was done. In the other three experiments, where the maize hybrid "Szegedi SC 444" with a medium stalk stability was grown, large differences of lodging could be observed. The extent of lodging in the plant stand (of the net plots) was evaluated before harvesting on October 12. For an estimation of the extent and degree of lodging, five categories were set up:

1 = plants standing $\pm$ 10° lodged,
2 = plants lodged to 10–30°,
3 = plants lodged to 30–50°,
4 = plants lodged to 50–70°,
5 = plants lodged to 70–90°.

It was observed that on each plot the plants could usually be grouped into 2 of the above categories. The category (1–5) numbers, when multiplied by the percentage of occurrence of a certain category (100% = 1) resulted in the weighed value of a given category, e.g. $(3 \times 0.7) + (5 \times 0.3) = 2.1 + 1.5 = 3.6$ means that on a certain plot 70% of the plant population were lodged to 30–50°, and 30% to 70–90°. The resulting sum indicates the degree of lodging in a certain plot. On each plot, the extent of critical lodging, i.e. the percentage of the plant population lodged to 70° or more, was also calculated.

Results and discussion

P- and K- status of the soil

The influence of the 12 years P- and K-fertilization on the AL-P- and AL-K-contents of the soil can be studied in Table 2. The differences in the AL-P- and K-supplies of the experiments "18" and "19" existing at the beginning of the experimental work, could also be detected in the 12th year of the experiments. However, as a consequence of P-fertilization the level of P-status — poor in the beginning — was increased to "medium" by the fertilizer doses 50 and 100 kg P_2O_5/ha/year, and to "good" by 150 kg/ha/year, while the P-doses of 200 kg/ha/year resulted in an overfertilization from the 12th year of the experiments. The AL-K_2O-contents of the unfertilized plots and of the plots fertilized with 100 kg K_2O/ha/year equally indicated a medium K-supply but the 200 kg K_2O/ha/year fertilizer doses resulted in a good K-supply of the soil. To raise the soil's AL-P_2O_5-content by 10 mg/kg in this experiment, 110–130 kg P_2O_5/ha was needed according to the calculations of Kádár (1983) based on the nutrient balance, i.e. on the quantity of nutrients remaining in the soil. At the same time, for the increase of the soil's AL-K_2O-content by 10 mg/kg, 270 kg K_2O/ha was needed, i.e. twice the quantity needed from P-fertilizers.

Early development of the plants, lodging due to windstorm and grain yield

As mentioned earlier, the maize was flowering when the storm of July 20, 1983, occurred. At that time, we already knew the status of development (determined by grading of the maize plants with 4–6 leaves), and we possessed data of earlier soil analyses, too. In Tables 3–5, data are summarized, which concern the status of development of maize plants with 4–6 leaves, the degree of lodging and the grain yields, harvested by hand, and "harvestable" by combine, as affected by NPK-fertilization. N-fertilization did not considerably influence the status of development of the plants with 4–6 leaves. However, with higher N-doses — as a tendency — rather a disadvantageous effect could be detected (Table 3). At the same time, the numbers indicating the degree of lodging were undoubtedly greater with higher N-doses (200–250 kg N/ha/year). The "critical" lodging (i.e. a lodging over 70°) — which makes harvesting by a combine-machine more difficult and/or causes heavy yield losses — is also given as percentages of the total plant stand: it can be seen that these numbers also increase with higher N-doses.

Surprisingly, high grain yields (7.4 t/ha in the average) could be received in 1983 when applying only 50 kg N/ha/year. Higher N-doses (200–250 kg/ha) caused a decrease in grain yield by 0.5–0.9 t/ha. "Harvestable" grain yield

Table 2

Effect of P- and K- fertilization on AL-P- and AL -K- contents after 12th year of the trials calcareous chernozem, Nagyhörcsök, 1980–1981

Code of trials	Time of sampling	P_2O_5 kg/ha/year						Average	K_2O kg/ha/year				Average
		0	50	100	150	200	$LSD_{5\%}$		0	100	200	$LSD_{5\%}$	
	In autumn:			AL-P_2O_5 mg/kg						AL-K_2O mg/kg			
B 1812	1980	73	89	119	173	218	33	134	170	183	210	16	188
A 1912	1981	106	134	182	227	293	36	188	166	198	228	74	197
B 1912	1981	107	125	181	232	299	31	189	160	194	254	27	203
Average		95	116	161	210	270		170	165	192	231		196
D		21	45	49	60		19	36	27	39		27	33

AL-P_2O_5 and -K_2O supply categories, according to the National Fertilizer Recommendation System

	low	moderate	good	extreme		low	moderate	good	extreme
AL-P_2O_5 mg/kg	<90	91–150	151–250	251<	AL-K_2O mg/kg	<130	131–200	201–300	301<

Table 3

Effect of nitrogen on the 4–6 leaf stage of development, on lodging due to windstorm, and on total (hand harvested) and "harvestable" grain yield

Szegedi SC 444 hybrid. (P_2O_5 = 100, K_2O = 100 kg/ha/year)

Trial	N kg/ha/year					$LSD_{5\%}$	Average
	50	100	150	200	250		
	Grading at 4–6 leaf age. June 6, 1983*						
A 1815	4.2	3.8	2.8	4.0	3.8	0.9	3.7
A 1914	4.8	4.0	4.0	3.5	3.5	1.0	4.0
B 1914	3.5	3.8	3.2	3.8	3.0	1.1	3.5
Average	4.2	3.9	3.3	3.8	3.4	0.7	3.7
	Degree of lodging**						
A 1815	1.6	1.5	2.1	2.9	2.8	1.3	2.2
A 1914	2.0	2.3	2.1	2.0	3.1	1.1	2.3
B 1914	1.6	2.2	1.6	2.6	2.5	1.2	2.1
Average	1.7	2.0	1.9	2.5	2.8	1.0	2.2
Critical lodging %***	6	4	3	14	17		9
	Grain yield, t/ha (total grain yield) (86% d.m.)						
A 1815	6.52	6.46	6.59	5.82	5.80	0.94	6.24
A 1914	7.94	7.11	8.02	7.62	7.00	1.12	7.54
B 1914	7.66	7.93	7.87	7.63	7.06	1.04	7.64
Average	7.37	7.17	7.49	7.02	6.62	0.67	7.14
	"Harvestable" grain yield, t/ha (by combine)**** (86% d.m.)						
A 1815	6.52	6.46	6.15	4.94	4.75	2.05	5.76
A 1914	7.94	6.20	8.02	7.62	4.99	2.04	6.95
B 1914	7.66	7.93	7.87	5.47	5.70	2.36	6.93
Average	7.37	6.86	7.35	6.07	5.15	1.24	6.55

* 1 = least developed; 5 = most developed;
** 1 = least lodged (fully standing); 5 = fully lodged (lying on the ground);
*** >70° lodging, in percentage of the population;
**** the yield of population less than 70° lodged.

was maximal at N 50 and 150 levels. At N 200 and 250 levels we received 1.3–2.2 t/ha less grain yield due to grain loss and increasing critical lodging. (We regarded plants lodged by more than 70° as not harvestable by combine.)

In Table 4 the influence of P-fertilizer on the same parameters is shown. Regular P-fertilization had a favourable influence on the status of development of maize plant with 4–6 leaves on this chernozem soil originally poor in P. Phosphorus exerted a favourable influence on the degree of lodging and on "critical" lodging, too: both values decrease as P-doses increase.

The grain yield of maize was strongly increasing with 50 kg P_2O_5/ha/year, and to a smaller degree, but still increasing, with 100 kg P_2O_5/ha/year.

Table 4

Effect of phosphorus on the 4–6 leaf stage of development, on lodging due to windstorm, and on total (hand harvested) and "harvestable" grain yield

Szegedi SC 444 hybrid. (N = 50–250; K_2O = 100 kg/ha/year)

Trial	P_2O_5 kg/ha/year					$LSD_{5\%}$	Average
	0	50	100	150	200		
	Grading at 4–6 leaf age. June 6, 1983*						
A 1815	2.7	4.2	3.7	4.0	4.1	0.5	3.7
A 1914	2.4	4.0	4.0	4.2	4.0	0.6	3.7
B 1914	2.0	3.3	3.4	4.0	3.8	0.7	3.3
Average	2.4	3.8	3.7	4.1	4.0	0.4	3.6
	Degree of lodging**						
A 1815	2.5	2.5	2.2	2.6	2.2	1.2	2.4
A 1914	2.6	2.5	2.3	2.1	1.6	0.8	2.2
B 1914	2.8	2.3	2.1	1.6	1.9	0.7	2.1
Average	2.6	2.4	2.2	2.1	1.9	0.6	2.2
Critical lodging %***	17	15	9	4	2		9
	Grain yield, t/ha (total grain yield) (86% d.m.)						
A 1815	4.78	6.05	6.24	5.47	5.04	0.52	5.51
A 1914	5.92	7.27	7.54	7.34	6.92	0.61	7.00
B 1914	5.75	7.51	7.63	7.23	6.65	0.58	6.95
Average	5.48	6.94	7.13	6.68	6.20	0.41	6.49
	"Harvestable" grain yield, t/ha (by combine)**** (86 d.m.)						
A 1815	4.37	5.16	5.76	5.20	5.04	1.48	5.11
A 1914	4.54	6.33	6.95	7.15	6.77	1.58	6.35
B 1914	4.62	6.25	6.92	7.23	6.45	1.44	6.30
Average	4.51	5.91	6.55	6.53	6.09	0.86	5.92

*, **, ***, ****: See in Table 3.

However, 150 and 200 kg P_2O_5/ha/year significantly reduced the grain yield by 0.4–1.0 t/ha. On this calcareous soil, poor in Zn, such a result can be explained by the P/Zn antagonisms which cause a depression in yield. The results of a plant analysis with diagnostic aim carried out in the same long-term experiment later on led to the same conclusions (Csathó et al. 1989), (Table 4). Considering "harvestable" yields, both P 100 and 150 levels gave the best results (> 6.5 t/ha).

As it is known, maize is a plant which demands much K. The advantageous influence of K on the early development of maize plants could be observed in this experiment, too (Table 5). The results of the unfertilized plants (no fertilization during the last 15 years) are also given in this table. As the differences in the nutrition status are the most obvious when the

Table 5

Effect of potassium on the 4–6 leaf stage of development, on lodging due to windstorm, and on total (hand harvested) and "harvestable" grain yield

Szegedi SC 444 hybrid

Trial	K_2O kg/ha/year					$LSD_{5\%}$	Average
	ø $N_0P_0K_0$	0 (N = 250, P_2O_5 = 150 kg/ha)	100	100 (N = 250, P_2O_5 = 200 kg/ha)	200		
	Grading at 4–6 leaf age. June 6. 1983*						
A 1815	1.0	2.0	3.8	3.8	5.0	0.9	3.1
A 1914	1.5	4.0	4.5	4.0	4.2	1.0	3.6
B 1914	2.0	3.0	3.5	3.8	4.8	1.1	3.4
Average	1.5	3.0	3.9	3.9	4.7	1.0	3.4
	Degree of lodging**						
A 1815	4.9	3.3	2.7	2.5	1.7	1.6	3.0
A 1914	4.1	4.4	2.4	1.4	1.4	0.8	2.7
B 1914	2.5	3.8	1.5	2.0	1.8	1.4	2.3
Average	3.8	3.8	2.2	2.0	1.6	0.6	2.7
Critical lodging %***	45	58	4	3	—		22
	Grain yield, t/ha (total grain yield) (86% d.m.)						
A 1815	3.82	4.95	5.58	5.08	4.64	0.94	4.81
A 1914	5.27	6.57	7.25	6.61	6.90	1.12	6.52
B 1914	5.68	5.50	7.18	6.44	6.19	1.04	6.20
Average	4.92	5.67	6.67	6.04	5.91	0.60	5.84
	"Harvestable" grain yield, t/ha (by combine)**** (86% d.m.)						
A 1815	0.57	2.62	5.16	5.08	4.64		3.61
A 1914	2.55	1.32	6.69	6.32	6.90	1.06	4.75
B 1914	5.68	3.19	7.18	6.06	6.19		5.66
Average	2.93	2.37	6.34	5.82	5.91	0.65	4.67

*, **, ***, ****: See in Table 3.

plants are young, the plants of the unfertilized plots were found — as could be expected — to be the least developed.

On the plots deficient in P (NKP_0), and on those overfertilized with N ($N_{250}P_{100}K_{100}$), the plants — as mentioned earlier — were lodged much more by the storm than on the other plots. However, lodging reached its highest degree on the unfertilized plots ($N_0P_0K_0$) and on those deficient in K (NPK_0). According to the indices used in this paper for the degree of lodging, number 5.0 would mean that the whole plant population was flattened to the ground. The indices 3.8 both for the plots $N_0P_0K_0$, and for the plots NPK_0 show the seriousness of the damage caused by the storm. The critical lodging was also the most severe on these plots, where 45% and 60%, resp., of the plant popu-

lation was lodged to 70° or more at harvest time. In agreement with data in literature, K prevented the greatest amount of lodging in this experiment also. The extent of lodging was diminished to an acceptable level by 100 kg K_2O/ha/year, and this K-dose prevented critical lodging, too. The extent of lodging was reduced further by the K-dose of 200 kg/ha/year: in this treatment the lowest lodging could be found.

The yield of the unfertilized plots was about 5 t/ha in the dry year of 1983. Fertilizing with NP increased the yield by 0.7 t/ha. As an effect of 100 kg K_2O/ha given yearly, the grain yield of maize increased by 1.0 t/ha on this chernozem soil initially supplied with a medium level of K. The application of 200 kg K_2O/ha/year resulted in no further increase of the yield (Table 5). Maximum "harvestable" yield was given also in the plots which received 100 kg/ha K_2O (83 kg/ha K) yearly. On the plots receiving no K fertilizer at all, "harvestable" yield was less than half of this.

As far as there was only a minimal interaction or none between NPK fertilization and N applied at different times, the effect of NPK fertilization on lodging and yield of maize is shown in the Tables 3–5 in the average of N-application at different times: However, when N-doses were divided evenly, applying one half in autumn and the other in spring before sowing, lodging increased and "harvestable" yields decreased as compared to the plots receiving the total amount of N in autumn (Fig. 1). The effect of dividing the N-doses into two halves was the most obvious in the K-control plots. N given in autumn can leach into deeper layers after a mild and wet winter, while spring application can be more effective. Dividing of N into two halves could increase the effectiveness of N application, but in this case lodging increased and the "harvestable" yield decreased, as can also be seen in Table 3 (Fig. 1).

Correlation among the status of development of maize plant with 4–6 leaves, the degree of lodging, and the grain yield

In these experiments a linear connection was found between the status of early development of plants (on the 6th June), and the extent of damage caused by the storm (Fig. 2). In the weakly developed plants the damage caused by the storm was more serious. The reason for this is probably because the weakly developed plants possess a weak root system, when compared to the plants well-supplied with NPK (Smith 1957, in Arnon 1975). However, the plants of the plots not fertilized with K ($N_3P_3K_0$) lodged to a much greater extent than it would be expected in regard to their status of development. As it is known, when the plants are deficient in K, the formation of large molecule carbohydrates from sugar-like carbohydrates is inhibited, and this effects the structure of the cell walls and the stability of the stalks as well.

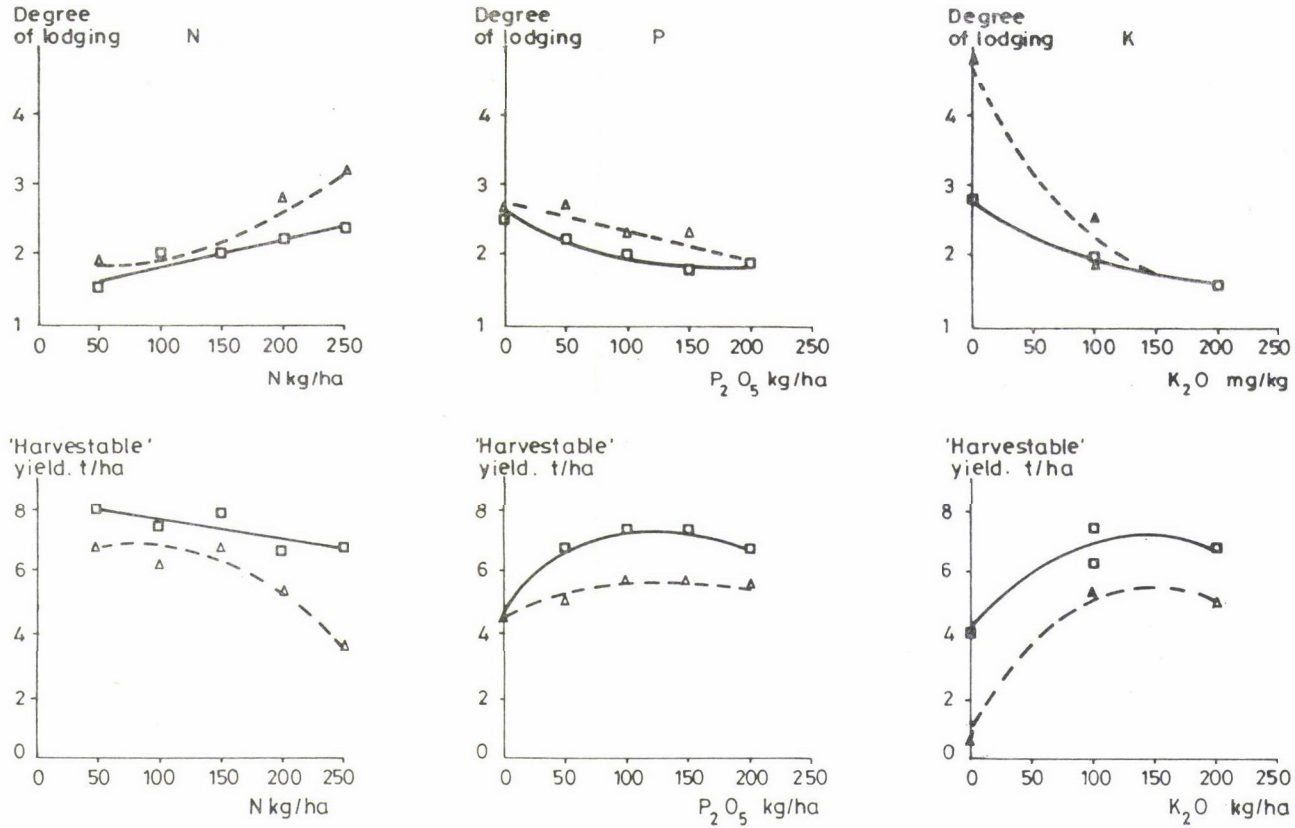

Fig. 1. Effect of NPK-fertilization and applying N at different times on lodging of maize due to windstorm and on "harvestable" grain yield. Szegedi SC 444. – – – – 1/2 N in autumn, 1/2 N in spring ——— whole N in autumn

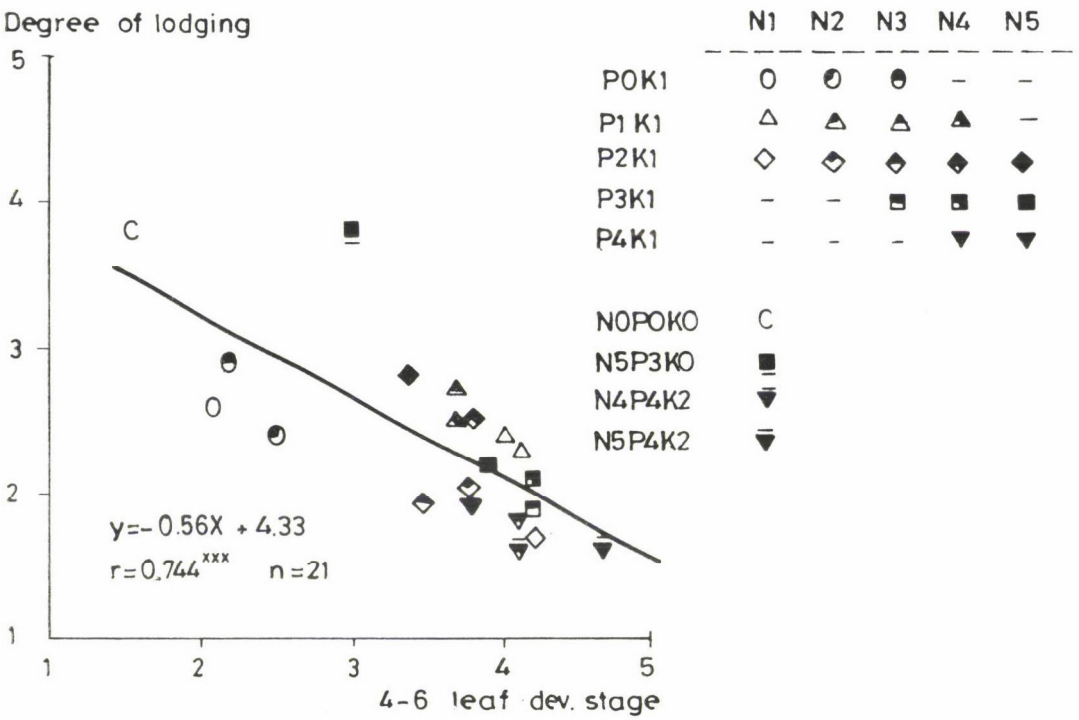

Fig. 2. Relationship between 4–6 leaf stage development of maize and lodging due to windstorm. Calcareous chernozem. Nagyhörcsök, Hungary. Szegedi SC 444 hybrid. 1983

However, no connection was found between the extent of lodging and the amount of grain yield. Grain yield was only affected by the nutrient supply.

In contrast to the other three experiments, in experiment B-1815 the maize hybrid "Pioneer SC 3901" was grown. This hybrid has outstanding stalk-stability and showed no lodging after the storm with any of the treatments. It can be supposed that this hybrid was able to make better use of the original nutrient content of the soil. The highest fertilizer doses over $N_{150}P_{50(100)}K_{100}$, however, decreased the yield of this hybrid, too. The decrease was about 0.5–1.0 t/ha, similarly to that of the hybrid "Szegedi SC 444" (Table 6).

When breeding and fertilizing maize we need both strong stalk hybrids with high potential yields and an adequate level of N, P and K fertilizers in the soils.

Table 6

Response to N, P and K fertilization in hybrid Pioneer SC 3901 Grain yield, t/ha (86% d.m.)

Trial	N, kg/ha/year (P_2O_5 = 100, K_2O = 100 kg/ha)					LSD5%	Average
	50	100	150	200	250		
B 1815	7.01	7.76	8.13	7.94	7.09	0.76	7.59

Trial	P_2O_5 kg/ha/year (N = 50–250, K_2O = 100 kg/ha)					LSD5%	Average
	0	50	100	150	200		
B 1815	7.04	7.43	7.59	6.85	6.94	0.76	7.17

Trial	ø $N_0P_0K_0$	K_2O kg/ha/year				LSD5%	Average
		0	100	100	200		
		(N = 250, P_2O_5 = 150kg/ha)		(N = 200–250, P_2O_5 = 200 kg/ha)			
B 1815	4.66	6.63	6.36	6.94	6.04	0.76	6.13

Acknowledgements

The author is thankful to Dr. J. Sarkadi for his valuable advice during the preparation of this paper, and also to Miss M. Gyimesi for her help in the mathematical and statistical elaboration of data.

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 295–317 (1991)

POTASSIUM FERTILIZATION IN HUNGARY: RESPONSES IN MAIZE AND IN OTHER CROPS

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(Received: 13 April, 1990; accepted: 20 June, 1990)

K-responses of maize in 1–10-year-long field fertilizer trials, set up in Hungary, found in literature between 1960 and 1989 are dealt with in the first part. In the second part the main findings of a 16-year-old long-term field K fertilizer experiment with different crops, carried out on a calcareous chernozem soil, moderately supplied with K, are reported.

The main findings are as the follows:

As a result of previous NPK experiment series in Hungary with cereals and row crops N-responses were highest in both groups, which was followed by the K-responses in row crops and P-responses in cereals. N-responses were twice as high as K- or P-responses.

Regarding the texture of Hungarian soils, 16% are sands, 10% sandy loams, 43% loams, 19% clay loams and 7% are clayey soils.

Soils with higher clay contents provided a better original (native) K-supplying power. Among clay minerals, usually smectite was dominant in heavy soils, and illite in light ones.

For estimating the extractable K-contents of the soils in Hungary, the AL-method (0.1 mol/L ammonium-lactate + 0.4 mol/L acetic acid, pH = 3.7, according to Egnér–Riehm–Domingo 1960) has been official since the 60s.

On the basis of K field experiments with maize between 1960 and 1989, the AL-method indicated the native K-supplying power of the soils quite accurately. By this method probably not only the K-adsorbed and in soil solution, but also some parts of fixed K was extracted.

The relationship between AL-K contents of K-control plots and relative yield of maize (yield in NP/NPK · 100, %) resembled a saturation-curve while connection between the AL-K contents of K-controls and surplus in maize (yield in NPK-NP, t/ha) could be described by a hyperbolic curve. (Fig. 2)

Average surplus in maize grain yields was 1.7 t/ha on sandy soils, 0.7 t/ha on sandy loams, 0.4 t/ha on loams, 0.2 t/ha on clay loams, and 0.1 t/ha on clayey soils. On the basis of K-response data in maize found in literature between 1960 and 1989, clay loams occurred only in medium, good, or extremely good native K-supplying categories, while clays in good and extremely good categories. On sandy soils, however, maize yielded up to 2.5–2.9 t/ha surplus; regular K-fertilization is thus suggested even for plants without expressed K-demands.

In our 16-year long-term field K-fertilizer trial which was set up on a calcareous chernozem soil, in Middle-West Hungary, originally moderately supplied with K, the following sequence was obtained in surpluses as a result of K-fertilization of the different crops:

potatoes (11.2 t/ha) > sugar beets (8.5 t/ha) > hemp (7.9 t/ha) > maize, 1976 (1.3 t/ha) > spring barley (0.7 t/ha) > winter wheat, 1975 (0.5 t/ha) > winter wheat 1974, maize 1977, winter barley (0.3–0.3 t/ha) > sunflower, poppy, oilflax, soybeans (0.2–0.2 t/ha) > mustard (0.1 t/ha) > rape (0.0 t/ha) > oats (−0.2 t/ha). The sequence in relative yield was as follows: potatoes (65%) < poppy (71%) < maize, 1976 (76%) < hemp (79%) < sugar-beet (85%) < spring barley (87%) < soybeans (89%) < winter wheat, 1975 (91%) < winter barley (92%) < oilflax (93%) < sunflower (94%) < winter wheat 1974, maize 1977 (95%) < mustard (97%) < rape (100%) < oats (104%).

In the calcareous chernozem soil originally moderately supplied with K (AL-K_2O on K-control: 140–160 mg/kg) it is advisable to raise the AL-K_2O contents to 200–220 mg/kg. Higher K-doses should be given for hoed plants with high K-demand (e.g. potatoes, sugarbeets, maize, hemp, etc.), while K-fertilization for cereals and oilplants can be reduced, or even stopped for a while within the rotation. When the previous plant is alfalfa with its high K-uptake, K-fertilization can be effective even for the latter plants.

The after-effect of initial 500–1000–1500 kg/ha K_2O (415–830–1245 kg/ha K) fertilization is long-lasting, and is measurable even in the 16th year, but diminishes with time. Maintaining K-fertilization of 100 and 200 kg/ha K_2O (83 and 166 kg/ha K) resulted in surpluses in the plots with initial K-application only after the 5–8th year, and mostly in plants greatly demanding K.

Keywords: K-response, maize, long-term field trials, Hungary

Introduction

Having no native potassium fertilizer industry, Hungary imports the total amount of this nutrient (mostly in the form of potassium chloride) needed for crop production.

In the first third of this century superphosphate was considered to be the most needed fertilizer in Hungarian agriculture (Cserháti–Kosutány 1887, Dorner 1924). However, in the field NPK fertilizer experiment series set up in the 40's and 50's, nitrogen fertilizer gave as much as two times more surplus in yield than did superphosphate. Potassium was the third among the macronutrients in increasing the yields of cereals (wheat, rye, barley, oats, etc.) and second best in increasing the yield of hoed plants (potatoes, maize) (Várallyay 1950, Sarkadi 1963, Latkovics 1963). Keresztény (1958) found good correlations between the logarithm of Nehring-K in control plots and the K-responses.

For estimating the available K- content in soil, the Nehring-method (0.2 mol/L NH_4NO_3) was used in Hungary from the 40's, which was replaced by the AL-method (0.1 mol/L ammonium lactate + 0.4 mol/L acetic acid, pH = 3.7) — (Egnér–Riehm–Domingo 1960) in the early 60's. This latter one has been official in Hungary for estimating the K-supply of the soils since that time. The number of soil tests increased together with the increasing use of fertilizers (Szabolcs 1969), and is obligatory for state farms and co-operatives in every 3rd year since 1977, and in every 5th year from 1990.

Hungary is in a favourable position regarding agricultural production. Climatic and soil conditions can help to achieve high yields if other factors (modern cultivars and hybrids, fertilizers, plant protection, etc.) are provided. The total area of the country is 93,000 km², of which 57% is arable land + gardens + vineyards + orchards; 13% is meadow + pastures (usually of poor status), 18% is forests and 12% is uncultivated (buildings, roads, etc.). Some fifty years ago the distribution of these groups was 64–18–12–6%, respectively. In Hungary the per capita agricultural area is over 0.6 ha, of

which 0.48 ha is arable land. This is 38% more than the world average and is twice as high as the average of the West European countries (Láng and Harnos 1985). One third of our agricultural products are usually exported.

The aim of this work is on one hand to summarize and evaluate the K-response data in maize found in field experiments set up in Hungary between 1960 and 1989. On the other hand, the main findings of a 16-year long-term field K-fertilization trial, set up in 1973, by Kádár, on a calcareous chernozem soil, moderately supplied with K, will be shown, using as test plants all the main crops grown in Hungary. The main results of the first 6–7 years of the experiment have already been published by Sarkadi (1979), Kádár et al. (1989) respectively Csathó and Kádár (1990).

Materials and methods

The K-response trials with maize in Hungary between 1960 and 1989

Elaborating the findings in literature, there were only included field trials using K-control (NP), and increasing rates of K-fertilizers on the same NP level. In this way probably neither the shortage of N, nor that of P limited the possible K-responses. Those trials were not included, where all the three N-, P- and K- doses were increased jointly and no K effect could be detected.

In Hungary KCl is almost the only potassium fertilizer form used (some K_2SO_4 is given to certain horticultural plants). In all the evaluated experiments 40 or 60% KCl was the K-source in fertilizers.

There were mostly found 2–4–8-year-old semi-long-term fertilizer trials of maize in the literature. In all the semi-long-term experiments the K-responses observed in the average of the years, were considered, to eliminate the 'year-effect'. The results of long-term trials more than 10 years old are not evaluated in this work. The following parameters were collected: AL-K_2O content of K_0 variants; upper limit of plasticity according to Arany; humus content; maize grain yield of K_0 variants; relative yield (NP : NPK yield ratio $\times$ 100); and surplus (NPK-NP yield, t/ha). Among the K-treatments the one chosen was that reaching maximum yield of at least 95% (maximum economic yield).

There were two restrictions made for the K-trials found in literature between 1960 and 1989: the results of the extremely dry year of 1968 were neglected, and also those of the experiments (two trials) were omitted, in which plant population density was lower, than 30 thousand plants/ha. The reason of the latter restriction was that, after the investigations of Berzsenyi-Janosits (1953), I'só (1958) and Győrffy (1962, 1979) it became evident that — also in Hungary — maize hybrids need high population density to show responses to fertilizers. In all the trials carried out between 1960 and 1989, hybrids were sown, there was not any trial with open-pollenated cultivars.

There were not found any trials of maize on certain sandy soils, with extremely poor humus status, because such soils are not suitable for this crop ("rye soils"). Neither were found maize trials on peat soils which are characterised in the literature as promoting great K-responses.

The set up of the 16-year-old long-term experiment on a chernozem soil

The 16-year-old factorial (4^3) long-term field experiment was set up by Kádár in autumn 1973, in Nagyhörcsök. The Nagyhörcsök Experimental Station of the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences s situated in Middle-West Hungary. The soil of the experimental station is a calcareous ihernozem soil, containing 3% humus and 5% $CaCO_3$. The AL-P_2O_5 and AL-K_2O contents cetermined by the method of Egnér–Riehm–Domingo, 1960 (0.1 mol/L ammonium lactate

+ 0.4 mol/L acetic acid, pH = 3.7) were 60–70 and 140–160 mg/kg soil, respectively. According to the official methods in Hungary, and the nutrient supply categories determined by these methods, the soil was originally very well supplied with Mn, satisfactorily with Mg and Cu, moderately with N and K, and poorly with P and Zn (MÉM NAK 1979, Kádár et al. 1989). As to the character of the soil texture, the soil of the experiment is a light loam, containing 20% sand, 40% loess-like powder, 20% silt and 20% clay. Among clay minerals, illite and chlorite are dominant. The parent rock is a 15–20 m thick loess, and ground water is at a 13 m depth.

The aim of the experiment was to examine the effectiveness of the different building-up and maintaining PK doses and their combinations on the yield of main crops and on extractable P-and K-contents of the soil. The number of the variants was 64, with 2 replications. In this paper the results of the K-experiment are reported, where the effect of the yearly applied 0–100–200 kg/ha K_2O (0–83–166 kg/ha K) was examined on the different initially given building-up K-levels (in the autumn of 1973 0–500–1000–1500 kg/ha K_2O, i.e. 0–415–830–1245 kg/ha K were applied). The layout of the experiment made it also possible to examine the 16-year-old after-effects of the initial K applications on the maintaining K = 0 level. On each plot 200 kg/ha N was given for crops demanding much N, and 100 kg/ha for crops needing smaller amounts of nitrogen. The average P added yearly was 75 kg/ha P_2O_5 (33 kg/ha P). During 16 years, the K-responses of most field crops cultivated in Hungary were investigated. The plants in the experiment were the following:
1974 and 1975: winter wheat (Kavkaz); 1976 and 1977: maize (MvSC 380); 1978: potatoes (Desirée); 1979: winter barley (Mv 35); 1980: oats (Leanda); 1981: sugarbeets (Beta Monopoly N-1); 1982: sunflower (Topflor-3); 1983: poppy (Kék Duna); 1984: rape (Yet Neuf); 1985: mustard (Budakalászi sárga); 1986: spring barley (Opal); 1987: oilflax (Szegedi 43); 1988: soybeans (Imola); 1989: hemp (Kompolti). Agrotechnics (ploughing, sowing, harvesting, etc.) common in agricultural practice in Hungary were used. Before beginning this experiment alfalfa was grown for 4 years, which absorbed high amounts of potassium from the soil.

The 50-year average precipitation was 590 mm/year on the experimental station. There was a drought in 1979 and 1983, while the years 1974, 1975 and 1984 were wetter than the average (Table 1).

Results and discussion

(A) *K-response trials of maize in Hungary between 1960 and 1989*

Some comprehensive works about the effectiveness and importance of potassium fertilization in Hungary were already published. Kovács summarized effectiveness of N-P-K fertilization on the basis of data of trials published in "Kísérletügyi Közlemények" between 1898 and 1947. Unfortunately, this report is not published. Fekete (1959) evaluated the results of 47 K-field trials with different plants. Hungarian soils were classified by Stefanovits and Sarkadi (1963) into 3 main groups according to their native (original) ability to supply crops with K (Fig. 1):

(1) On saturated or only slightly leached soils which developed either on alluvial soils containing large amounts of mica, or on weathered volcanic rock, or on loess, winter cereals respond quite seldom to K, while crops demanding much K (e.g. sugarbeets) may respond to K-fertilizers.

(2) On brown forest soils, on rustbrown forest soils developed on Pannonian deposits (sediments), on chernozem brown forest soils and on lighter chernozem soils, crops having a high K-demand (sugarbeets, potatoes, hemp, tobacco, spring barley for beer, maize, papilionaceae, vegetables, grapes,

Table 1

Distribution of precipitation at Nagyhörcsök Research Station, Middle West Hungary, 1973–1989

Months	1973/74	1974/75	1975/76	1976/77	1977/78	1978/79	1979/80	1980/81
Oct.	35	208	70	99	16	33	27	56
Nov.	39	35	22	47	79	11	74	152
Dec.	90	41	30	49	26	48	68	39
Sum	164	283	122	195	121	92	169	247
Jan.	37	18	25	30	8	66	33	8
Febr.	55	9	4	63	24	48	19	15
March	12	36	44	52	36	13	22	34
Sum	104	63	73	145	68	127	74	57
April	34	39	56	35	42	50	53	6
May	93	86	20	49	75	10	41	45
June	131	147	37	40	119	50	63	101
Sum	258	272	113	124	236	110	157	152
July	50	104	39	33	107	44	31	42
Aug.	82	182	77	62	10	65	71	53
Sept.	98	41	95	36	31	19	22	40
Sum	230	327	211	131	148	128	124	135
Total	756	945	519	595	573	457	524	591

Months	1981/82	1982/83	1983/84	1984/85	1985/86	1986/87	1987/88	1988/89	50 years average
Oct.	22	37	42	54	16	54	8	27	53
Nov.	34	19	32	54	97	10	78	14	57
Dec.	115	55	10	39	57	33	28	38	42
Sum	171	111	84	147	170	97	114	79	152
Jan.	31	35	63	8	41	66	38	6	34
Febr.	19	47	32	50	33	12	53	24	36
March	39	33	22	58	49	54	58	42	37
Sum	89	115	117	116	123	132	149	72	107
April	41	23	33	23	43	58	25	72	48
May	29	105	75	55	53	86	11	44	64
June	72	14	48	87	78	68	70	62	61
Sum	142	142	156	165	174	212	106	178	173
July	88	19	23	24	19	26	30	65	54
Aug.	50	51	61	77	26	74	97	78	55
Sept.	15	10	115	11	—	44	57	1	49
Sum	153	80	199	112	45	144	184	144	158
Total	555	447	672	540	512	585	553	473	590

fruit trees, etc.) respond quite often to K-fertilization, and K-application can be effective even in winter cereals.

(3) The native K-content of wind blown sands, sands, sandy rustbrown forest soils with clay illuviation is small, so it is necessary to add potassium regularly not only for K-demanding crops, but also for cereals.

(4) Uncultivated area (Fig. 1).

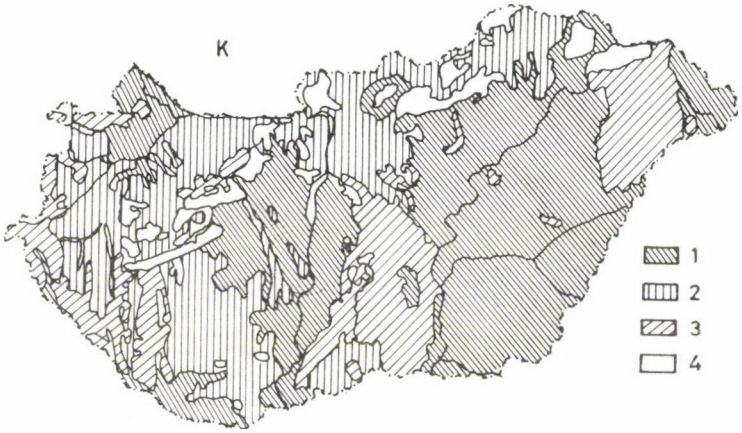


Fig. 1. The map of predictable K-responses in Hungary (native K-supply power of the soils) Stefanovits and Sarkadi, 1963. Note: see in text

On the basis of foreign and Hungarian papers, Láng (1963) reviewed the role of potassium in the plants, and the factors influencing the effectiveness of K-application.

Kádár (1980) gave a historical outline of the role of K in the increase of soil fertility. He determined also the K-balance of Hungary between 1932 and 1984 (Table 2), which is positive since the late 60's, and from the middle 70's it lies between +40 and +50 kg/ha/year K_2O.

From the late 80's there has been a decrease in K (and P) consumption in Hungarian agriculture.

On the basis of data of K-fertilizer trials with maize carried out in the last 30 years in Hungary, the purpose of this paper is to summarize and evaluate the K-responses of maize on typical Hungarian soils. Maize was chosen because of its increased demand for K (although potatoes and sugarbeets are needing even more of potassium) and because it is the second main crop (after winter wheat) cultivated in Hungary, (it is produced on about 25% of arable land, i.e. on ~ 1,150 thousand hectares). At the same time, potatoes and sugarbeets are grown only on 50 and 100 thousand hectares, respectively, and their importance in the national economy is a degree smaller than that of maize.

Table 2

K_2O-balance in Hungary, 1932–1984 (agricultural area, kg/ha)
Kádár, 1980 and 1987

Heading of K_2O-balance	1932–36	1960–64	1971	1975	1984
Uptake by yield	38	48	61	76	84
Supplied					
by manures	16	18	20	21	30
by fertilizers	—	7	45	82	71
by by-products	—	—	17	25	24
Total	16	25	82	128	125
Balance	−22	−23	21	52	41
*Intensity of the saldo, %	42	52	134	168	149

* Quotient shows how much percentage of the K-uptake had been returned by fertilization.

Searching for data of K-experiments with maize, several hundred individual trials were found in the last 30 years of Hungarian literature. The duration of these trials were 2–4–8 years with maize monoculture or wheat-maize–maize-wheat diculture. To minimize the "year-effects" the individual (yearly) data of a certain long-term trial are expressed in the 2–4–8 year average to receive more stable K-responses in that place. Altogether 41 experiments showing the K-responses in an average of less than 10 years, were formed in this way out of the several hundred individual trials. The plant population density was usually 31–48 thousand plants/ha at the beginning, and 56–71 thousand plants/ha at the end of the period. The basic N and P given was mostly between 120 and 160 kg/ha N and 60 and 100 kg/ha P_2O_5.

On the basis of K trial data of maize found in Hungarian literature between 1960 and 1989, a relationship between the AL-K_2O content (Egnér–Riehm–Domingo 1960) of K-control (NP) plots and the relative yield (yield in NP/yield in NPK · 100) and between the AL-K_2O in K-control plots and surplus (yield in NPK-yield in NP, t/ha) is expressed in Fig. 2. The AL-K_2O contents in the K-control plots refer to the native (original) K-supplying power of the soils, because in the experimental fields there was no previous K-fertilization, or it occurred only to a small extent. The highest K-responses (i.e. smallest relative yields) were observed with maize the sandy soils, originally poor in K. The K-responses were less in sandy loams, loams, and were minimal in clay loam and clay soils. In the latter case sometimes there was no K-responses in the maize at all. The connection between the AL-K_2O contents of K-control plots and the relative yields is described by a saturation curve, i.e. the soil originally better supplied with K shows smaller K-responses (Fig. 2 A). The changes in soil texture (the increase of clay content

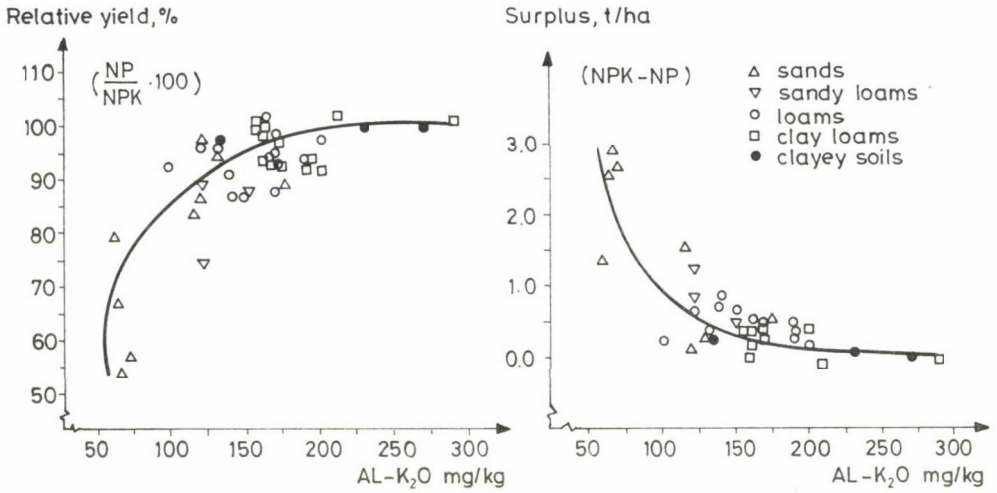


Fig. 2. Relationship among the AL-K_2O content of control plots, the relative yield and the surplus of maize in trials carried out in Hungary between 1960 and 1989

of the soils) were followed by the increase of the AL-K contents, too. As it is seen in the K-response curves, the AL-K contents characterised the K-supply of the soils quite accurately. Füleky (1987) reports that in pot experiments with ryegress (6 cuts) on typical soils of Hungary the best connection between the K-uptake of the plants and the soil K-tests was in the case of the AL-method ($r = 0.86$), showing better correlations than EUF ($r = 0.82$) and Mehlich ($BaCl_2$) ($r = 0.71$)-methods. On the basis of K-response data of maize in the last 30 years, no clay loam and clay soils were found with originally poor or even medium K-supply categories (i.e. showing large K-responses). The soils supplied maize with potassium well or very well without any K-fertilization. This is possible not true for potatoes and sugarbeets, which need even more potassium than does maize. The small number of trials on those soils is to be considered also (Figure 2 A).

The connection between the AL-K contents of the soils and the surplus in maize grain yields (yield in NPK-NP, t/ha) was found to be hyperbolic (Fig. 2 B). In sandy soils originally poor in K-surpluses in maize grain yield were obtained up to 2.5–2.9 t/ha, while no surplus, or a minimal one, was received in clay soils.

In Table 3, the effect of K-fertilization is shown on soils with different soil texture and other soil properties. If K_A (i.e. the upper limit of plasticity, according to Arany) is < 30: sand; 31–37: sandy loam; 38–42: loam; 43–50: clay loam; > 51: the soil is a clayey soil (Stefanovits 1971). Humus content and AL-K increase together with the clay content of the soils. To reach maximum economic yields of maize, there was 70–130 kg/ha more K_2O fertilizer

Table 3

K-responses of maize in field trials in Hungary between 1960 and 1989, in soils of different textures

Soil texture	Number of averaged trials	K_A	Humus %	AL-K_2O mg/kg in K-control	K_2O dose kg/ha	Yield in K-control t/ha	Relative yield, %	Surplus t/ha
Sands	8	28	1.20	83	130	4.68	74	1.68
Sandy loams	5	36	2.14	134	134	5.56	88	0.69
Loams	14	40	2.68	156	59	5.43	94	0.36
Clay loams	11	45	2.92	184	34	5.71	96	0.16
Clayey soils	3	56	3.27	210	—	7.06	100	—

needed in sandy and sandy loam soils than in soils with higher amounts of clay. The grain yield in K-control (NP) plots and the relative yield are higher in heavier soils than in light ones. Surpluses change in the opposite direction. Nevertheless, K-responses can also be affected by not only the soil properties in the ploughed layer, but by other characteristics (e.g. the depth of ground water from the surface, depth of the humus layer, obstructive layers, etc.), too. According to soil texture, 16% of the soils in Hungary are sandy soils, 10% are sandy loams, 43% are loams, 19% are clay loams and 7% are clays (Várallyay et al. 1980). On loams, representing the largest part of soils in Hungary, the average surplus was 0.2–0.8 t/ha in maize caused by the effect of K-fertilization in the field experiments carried out in the last 30 years.

Among the research sites on 14 soils, there are also known clay mineral composition data (Table 4). On the basis of field K-response data of maize between 1960 and 1989 there was no connection observed between the clay mineral composition and K-responses. With the same clay contents, however, the number of the soils was not sufficient to make a reliable comparison in this regard.

As a result of positive K-balance saldo in the last 15–20 years, the AL-K content of Hungarian soils has been increasing since that time. Prior to the intensive use of (NP)K fertilizers, about half of the soils were poorly or moderately supplied with potassium (Stefanovits and Sarkadi 1963), while in 1987, according to Buzás et al. (1988) the area of such soils was diminished to 1/3 of the arable land. Figure 3 shows the average AL-K_2O contents of Hungarian soils in 1984—1985 (Baranyai, Fekete, Kovács, in: Sarkadi and Várallyay 1989). According to AL-K_2O supply categories elaborated on the basis of K-response data of maize trials carried out between 1960 and 1989 (Table 5), we have large areas where probably even maize with its high K-demand, would not answer (with surplus in yield) to K-fertilization. In such areas K-application should and can be stopped for years without any

Table 4

Relationship between clay content, clay mineral composition, and K-response in maize
(Clay content, clay mineral composition data: Stefanovits and Rózsavölgyi, in: Füleky, 1987)

	Clay % (2 μm)	Clay mineral composition, %								Relative yield, %	Surplus (yield of NPK-NP, t/ha)
		Ill	Ka	Cl	Sm	Ve	Ill-Sm	Ill-Cl	Ill-Ve		
1. Őrbottyán	5.4	50.4	—	22.5	13.6	—	13.5	—	—	65	2.33
2. Nagykanizsa	15.7	58	4	16	6	2	9	5	—	89	0.66
3. Keszthely	21.9	59	10	13	6	—	9	3	—	90	0.64
4. Nagyhörcsök	23.1	47	—	29	16	—	5	3	—	90	0.59
5. Iregszemcse	24.3	50	—	30	8	—	10	2	—	87	0.83
6. Mosonmagyaróvár	25.4	48	—	28	16	—	7	—	—	96	0.24
7. Orosháza	27.6	43	—	19	10	6	18	3	1	101	−0.06
8. Szeged-Öthalom	29.1	64	12	20	—	—	—	4	—	94	0.26
9. Martonvásár	30.3	52	—	9	11	11	8	4	5	94	0.30
10. Putnok	31.3	33	14	—	27	—	19	—	2	95	0.32
11. Hajdúböszörmény	36.3	29	—	7	47	6	5	3	3	97	0.23
12. Szarvas	36.8	21	8.3	—	64.1	—	6.6	—	—	99	0.06
13. Karcag	44.8	56	—	17	7	3	11	5	1	102	−0.12
14. Kompolt	46.0	27	20	—	37	—	10	6	—	100	0.02

Ill = illite, Ka = kaolinite, Cl = chlorite, Sm = smectite, Ve = vermiculite

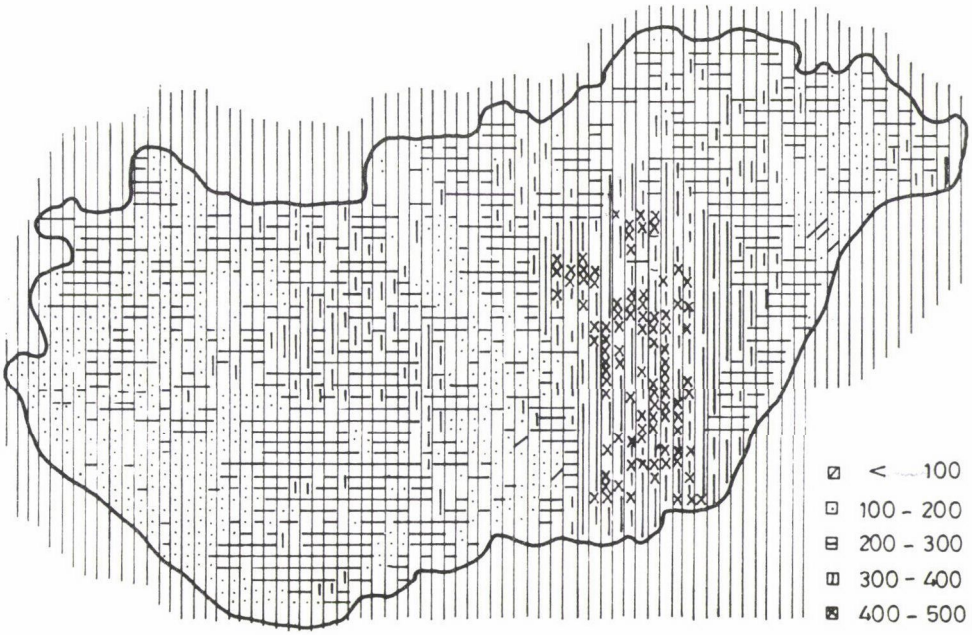


Fig. 3. Average AL-K_2O contents of Hungarian soils, in 1984 (Baranyai–Fekete–Kovács, in: Várallyay, 1989)

loss in yield. Overfertilization with potassium is not economic, and can promote leaching of important nutrients (Ca, Mg, etc.) into deeper layers (Kádár 1988, Stefanovits 1985). In sandy soils originally poor in K, however, even cereals (wheat, barley, rye, oats, etc.) could react to a yearly application of K. If new data of K experiments are obtained, the AL-K_2O supply categories can be modified according to the new findings.

Table 5

AL-K_2O supply categories for maize in different soil textures as estimated from the K-respons data in field experiments between 1960 and 1989

Soil texture	AL-K_2O supply categories, mg/kg				
	very low	low	moderate	good	extreme
Sands	< 60	61–90	91–120	121–150	151<
Sandy loams	<100	101–140	141–170	171–200	201<
Loams	<120	121–150	151–180	181–220	221<
Clay loams	<130	131–160	161–190	191–240	241<
Clayey soils	<140	141–170	171–200	201–260	261<

Table 6

Connection between the after-effects of initial (build-up) and the effects of yearly (maintenance) application of K on the main yields
Calcareous chernozem, Nagyhörcsök, 1974–1989
(Sarkadi, 1979; Kádár–Csathó–Sarkadi, 1989)

Year	Crop	Initial K_2O appl. in autumn 1973, kg/ha	Maintenance K_2O, kg/ha/year						
			0	100	200	0	100	200	$LSD_{5\%}$
			Main yield (grain, tuber, root) in t/ha						
						After-effect	Effect		
1973/74	Winter wheat (Kavkaz)	0	5.6	—	—	—	—	—	0.4
		500	5.8	—	—	0.2	—	—	
		1000	5.9	—	—	0.3	—	—	
		1500	6.0	—	—	0.4	—	—	
		$LSD_{5\%}$				0.4			
1974/75	Winter wheat (Kavkaz)	0	4.9	5.0	5.3	—	0.1	0.4	0.3
		500	5.2	5.3	5.2	0.3	0.1	0.0	
		1000	5.4	5.4	5.4	0.5	0.0	0.0	
		1500	5.5	5.4	5.6	0.6	−0.1	0.1	
		$LSD_{5\%}$					0.3		
1975/76	Maize (Mv SC 380)	0	4.2	5.2	4.8	—	1.0	0.6	0.6
		500	5.5	5.2	5.3	1.3	−0.3	−0.2	
		1000	5.5	5.4	5.3	1.3	−0.1	−0.2	
		1500	5.2	5.4	5.2	1.0	0.2	0.0	
		$LSD_{5\%}$					0.6		
1976/77	Maize (Mv SC 380)	0	8.2	8.3	8.1	—	0.1	−0.1	0.5
		500	8.6	8.4	8.5	0.4	−0.2	−0.1	
		1000	8.6	8.8	8.3	0.4	0.2	−0.3	
		1500	8.7	8.8	8.5	0.5	0.1	−0.2	
		$LSD_{5\%}$					0.5		
1977/78	Potatoes (Desirée)	0	20.5	26.2	31.7	—	5.7	11.2	2.8
		500	26.4	27.7	32.2	5.9	1.3	5.8	
		1000	29.5	30.3	30.8	9.0	0.8	1.3	
		1500	30.2	30.6	30.0	9.7	0.4	−0.2	
		$LSD_{5\%}$					2.8		
1978/79	Winter barley (Mv 35)	0	3.7	3.8	4.0	—	0.1	0.3	0.3
		500	3.8	3.9	3.9	0.1	0.1	0.1	
		1000	3.8	3.8	4.0	0.1	0.0	0.2	
		1500	3.8	3.9	3.9	0.1	0.1	0.1	
		$LSD_{5\%}$					0.3		
1979/80	Oats (Leanda)	0	5.4	5.1	5.2	—	−0.3	−0.2	0.6
		500	5.2	5.3	5.3	−0.2	0.1	0.1	
		1000	5.2	5.3	5.4	−0.2	0.1	0.2	
		1500	5.3	5.2	5.1	−0.1	−0.1	−0.2	
		$LSD_{5\%}$					0.6		
1980/81	Sugarbeets (Beta MN1)	0	47.3	50.5	55.8	—	3.2	8.5	4.5
		500	53.3	54.3	56.0	6.0	1.0	2.7	
		1000	55.2	50.9	57.2	7.9	−4.3	2.0	
		1500	53.7	55.8	53.1	6.4	2.1	−0.6	
		$LSD_{5\%}$					4.5		

Table 6 (cont'd)

Year	Crop	Initial K_2O appl. in autumn 1973, kg/ha	Maintenance K_2O, kg/ha/year						LSD5%
			0	100	200	0	100	200	
			Main yield (grain, tuber, root) in t/ha						
						After-effect	Effect		
1981/82	Sunflower (Topflor-3)	0	3.1	3.3	2.9	—	0.2	−0.2	0.4
		500	3.1	3.0	2.9	0.0	−0.1	−0.2	
		1000	3.1	3.1	2.9	0.0	0.0	−0.2	
		1500	3.2	3.0	3.0	0.1	−0.2	−0.2	
		$LSD_{5\%}$					0.4		
1982/83	Poppy (Kék Duna)	0	0.6	0.7	0.8	—	0.1	0.2	0.1
		500	0.7	0.7	0.8	0.1	0.0	0.1	
		1000	0.7	0.7	0.8	0.1	0.0	0.1	
		1500	0.7	0.8	0.7	0.1	0.1	0.0	
		$LSD_{5\%}$					0.1		
1983/84	Rape (Yet Neuf)	0	2.0	2.0	1.8	—	0.0	−0.2	0.4
		500	1.9	1.9	2.1	−0.1	0.0	0.2	
		1000	1.9	1.7	2.1	−0.1	−0.2	0.2	
		1500	1.8	1.9	1.9	−0.2	0.1	0.1	
		$LSD_{5\%}$					0.4		
1984/85	Mustard (Budakalászi sárga)	0	2.4	2.4	2.5	—	0.0	0.1	0.3
		500	2.3	2.4	2.6	−0.1	0.1	0.2	
		1000	2.6	2.5	2.4	0.2	−0.1	−0.2	
		1500	2.4	2.5	2.7	0.0	0.1	0.3	
		$LSD_{5\%}$					0.3		
1985/86	Spring barley (Opal)	0	4.7	5.3	5.4	—	0.6	0.7	0.3
		500	5.0	5.2	5.5	0.3	0.2	0.5	
		1000	5.1	5.1	5.5	0.4	0.0	0.4	
		1500	5.0	5.2	5.4	0.3	0.2	0.4	
		$LSD_{5\%}$					0.3		
1986/87	Oilflax (Szegedi 43)	0	1.6	1.8	1.8	—	0.2	0.2	0.2
		500	1.8	1.9	1.7	0.2	0.1	−0.1	
		1000	1.7	1.8	1.8	0.1	0.1	0.1	
		1500	1.7	1.9	1.7	0.1	0.2	0.0	
		$LSD_{5\%}$					0.2		
1987/88	Soybeans (Imola)	0	1.8	2.0	1.8	—	0.2	0.0	0.3
		500	1.9	2.0	1.6	0.1	0.1	−0.3	
		1000	1.9	1.9	1.7	0.1	0.0	−0.2	
		1500	1.9	1.9	1.6	0.1	0.0	−0.3	
		$LSD_{5\%}$					0.3		
1988/89	Hemp (Kompolti)	0	29.2	35.8	37.1	—	6.6	7.9	6.1
		500	31.8	33.4	37.4	2.6	1.6	5.6	
		1000	30.8	39.0	33.0	1.6	8.2	2.2	
		1500	32.2	38.5	37.1	3.0	6.3	4.9	
		$LSD_{5\%}$					6.1		

(B) *Results of a 16-year-old K-fertilizer trial on a calcareous chernozem soil*

The effect of potassium fertilization on the yields

On a calcareous chernozem soil, originally moderately supplied with K, in an experiment set ut by Kádár in autumn 1973, the effectiveness of maintaining K doses beside different initial K-levels was studied with the main crops cultivated in Hungary (Sarkadi 1979, Kádár et al. 1989, Csathó and Kádár 1990) (Table 6). The initial building-up of K-application was 0–500–1000–1500 kg/ha K_2O (0–415–830–1245 kg/ha K), and the yearly doses of maintaining K-fertilization were 0–100–200 kg/ha K_2O (0–83–166 kg/ha K). In the treatments with no maintaining K-application (fresh K = 0 kg/ha) it was also possible to study the 16-year after-effect of initial K-applications on the different cultivated plants. Between 1974 and 1989, on this soil originally moderately supplied with potassium, potatoes (+11.7 t/ha: 57%), sugarbeets (+9.9 t/ha: 21%), hemp (+8.2 t/ha: 27%) and maize in the relatively dry year of 1976 (+1.3 t/ha: 31%) responded mostly to K-fertilization. These crops are known as species demanding K exceedingly. In the wet year of 1977, however, maize yields were high, but the surplus (0.5 t/ha: 6%) was not expressed. It is generally known that nutrient responses can be more expressed in relatively dry years, than in wet ones. Among cereals and oil plants, winter wheat (in 1975), spring barley and poppy showed moderate, but significant K-responses (0.2–0.8 t/ha: 12–42%). In the other crops no significant response, or even no surplus at all was observed (Table 6).

In the first years the after-effects of initial (building-up) K-fertilizer were higher than the effects of maintaining K-fertilization of the initial K = 0 kg/ha level. However, from the 5–8th years of the experiment K-after-effects began to diminish, the cumulative effect of fresh K-application gradually exceeded them. To summarize the yearly application of 100 kg/ha K_2O (83 kg/ha K) up to 1979: 500 kg/ha, up to 1984: 1000 kg/ha, up to 1989: 1500 kg/ha K_2O was given. The effects of fresh K and the after-effect of initial K-application could be compared exactly on the basis of mutual K-balance saldo intervals. The after-effect of 500 kg/ha initial K_2O or the effects of the 100 kg/ha maintaining K_2O doses supplied the plants satisfactorily except for the most K-demanding plants, i.e. potatoes, sugarbeets, and hemp.

The effect of K-fertilization on the AL-K_2O contents of the soil

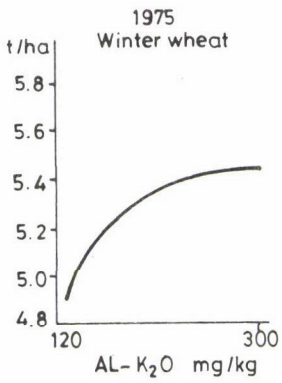
The doses of 500–1000–1500 kg/ha initial K_2O (415–830–1245 kg/ha K) application increased the AL-K_2O contents by 65–151–230 mg/kg (Table 7). These differences decreased up to 1980 to 29–39–49 mg/kg, and up to 1986 to 16–42–44 mg/kg, respectively, by the mutual effect of K-fixation and K-uptake by crops (the by-products were removed from the plots in the experi-

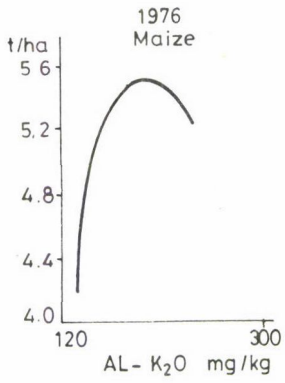
Table 7

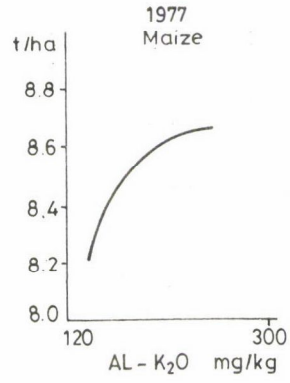
The after-effect of initial (build-up) and the effect of maintenance K-fertilization on the AL-K_2O contents of the soil

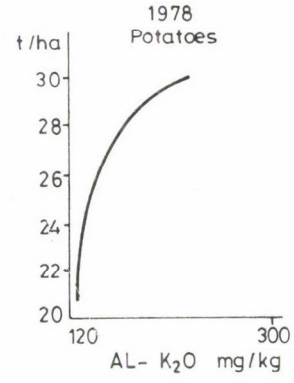
Calcareous chernozem, Nagyhörcsök, 1974–1986

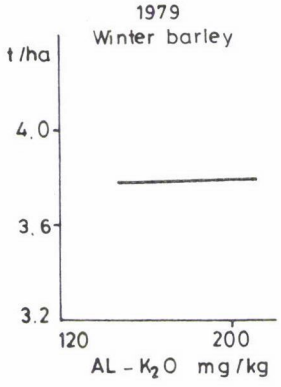
Years	Initial (build-up) K_2O kg/ha on autumn 1973	Maintenance K_2O kg/ha/year					Maintenance K_2O kg/ha/year				
		0	100	200	$LSD_{5\%}$	Mean	0	100	200	$LSD_{5\%}$	Mean
		AL-K_2O, mg/kg					$K_0 = 100\%$				
1974	0	128	128	125		127	100	100	98		99
	500	193	203	180		192	151	159	141	29	150
	1000	279	297	293	37	289	218	232	229		226
	1500	358	394	346		366	280	308	270		286
	$LSD_{5\%}$		37			16		29			12
	Mean	240	256	236	18	244	187	200	184	14	190
1976	0	138	152	188		152	100	110	136		115
	500	170	188	199	49	181	123	136	144	36	134
	1000	219	226	240		223	159	164	174		166
	1500	235	278	312		265	170	201	226		199
	$LSD_{5\%}$		49			22		36			16
	Mean	190	210	234	23	205	138	153	170	17	154
1978	0	127	144	172		139	100	113	135		116
	500	145	157	210	39	159	114	124	165	31	134
	1000	173	208	241		195	136	164	190		163
	1500	225	246	266		233	177	194	209		193
	$LSD_{5\%}$		37			17		29			13
	Mean	168	189	222	21	181	132	149	175	16	152
1980	0	167	191	239		186	100	114	143		119
	500	196	221	259	30	209	117	132	155	18	135
	1000	206	233	290		223	123	140	174		146
	1500	216	257	282		241	129	154	169		151
	$LSD_{5\%}$		30			13		18			8
	Mean	196	225	267	17	215	117	135	160	10	138
1982	0	156	197	234		196	100	126	150		125
	500	179	191	215	37	195	115	122	138	24	125
	1000	189	216	282		229	121	138	181		147
	1500	193	244	228		222	124	156	146		142
	$LSD_{5\%}$		40			23		26			15
	Mean	179	212	240	17	210	118	136	154	11	135
1986	0	130	206	274		203	100	158	211		156
	500	146	212	260	36	206	112	163	200	28	158
	1000	172	229	304		235	132	176	234		181
	1500	174	242	303		240	134	186	233		184
	$LSD_{5\%}$		30			16		23			12
	Mean	156	222	285	26	221	120	171	220	20	170

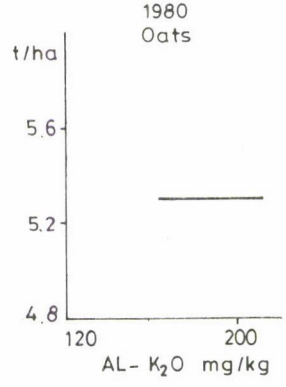

1975
Winter wheat
t/ha
5.8
5.6
5.4
5.2
5.0
4.8
120
300
AL- K2O mg/kg

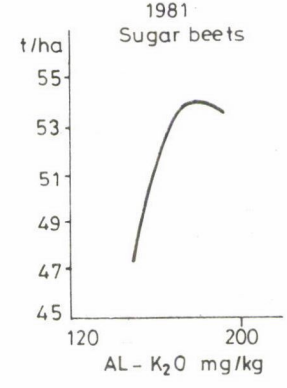

1976
Maize
t/ha
5.6
5.2
4.8
4.4
4.0
120
300
AL- K2O mg/kg

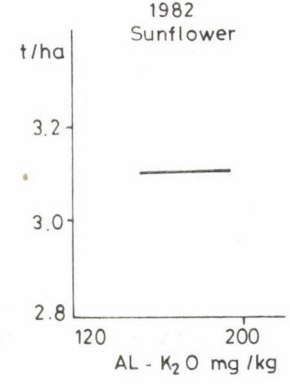

1977
Maize
t/ha
8.8
8.6
8.4
8.2
8.0
120
300
AL- K2O mg/kg


1978
Potatoes
t/ha
30
28
26
24
22
20
120
300
AL- K2O mg/kg


1979
Winter barley
t/ha
4.0
3.6
3.2
120
200
AL - K2O mg/kg


1980
Oats
t/ha
5.6
5.2
4.8
120
200
AL- K2O mg/kg


1981
Sugar beets
t/ha
55
53
51
49
47
45
120
200
AL- K2O mg/kg


1982
Sunflower
t/ha
3.2
3.0
2.8
120
200
AL - K2O mg/kg

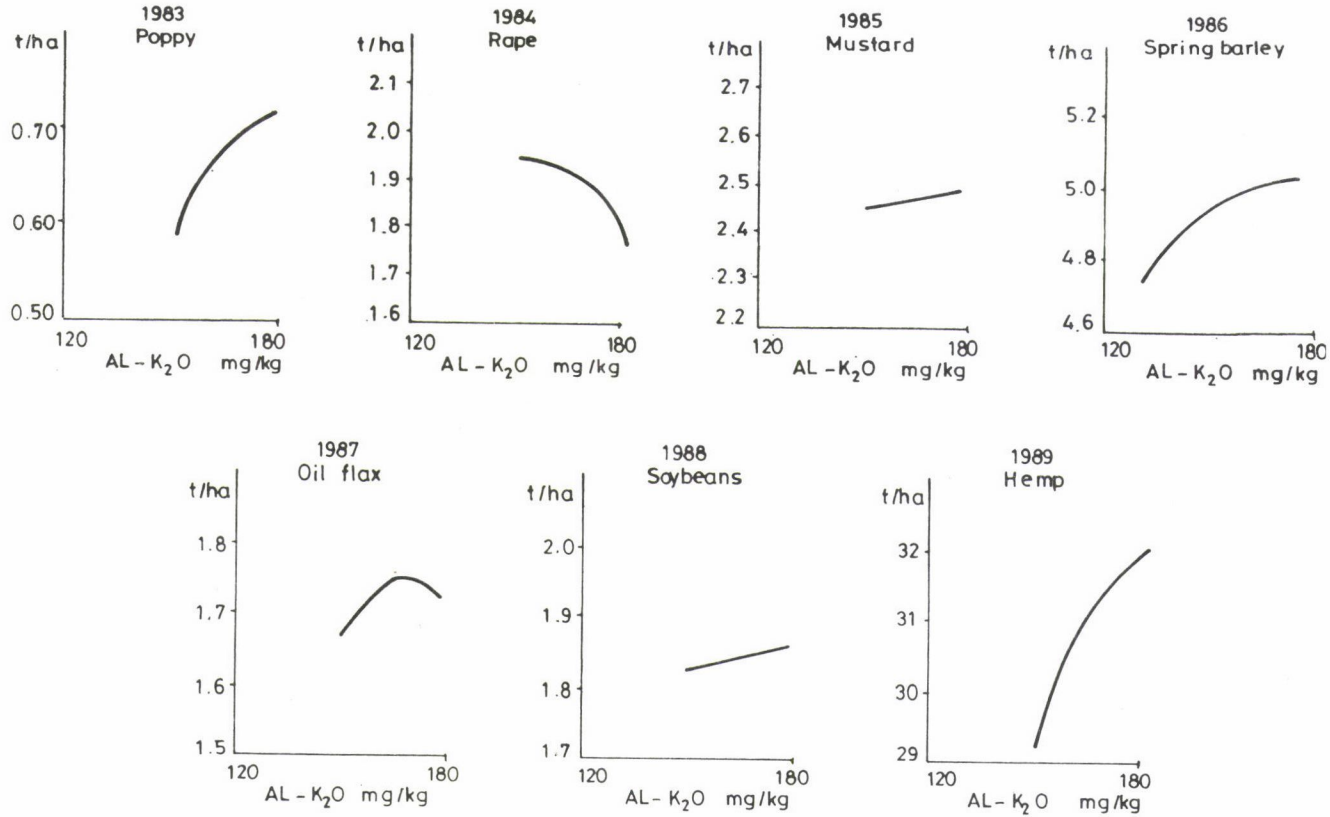
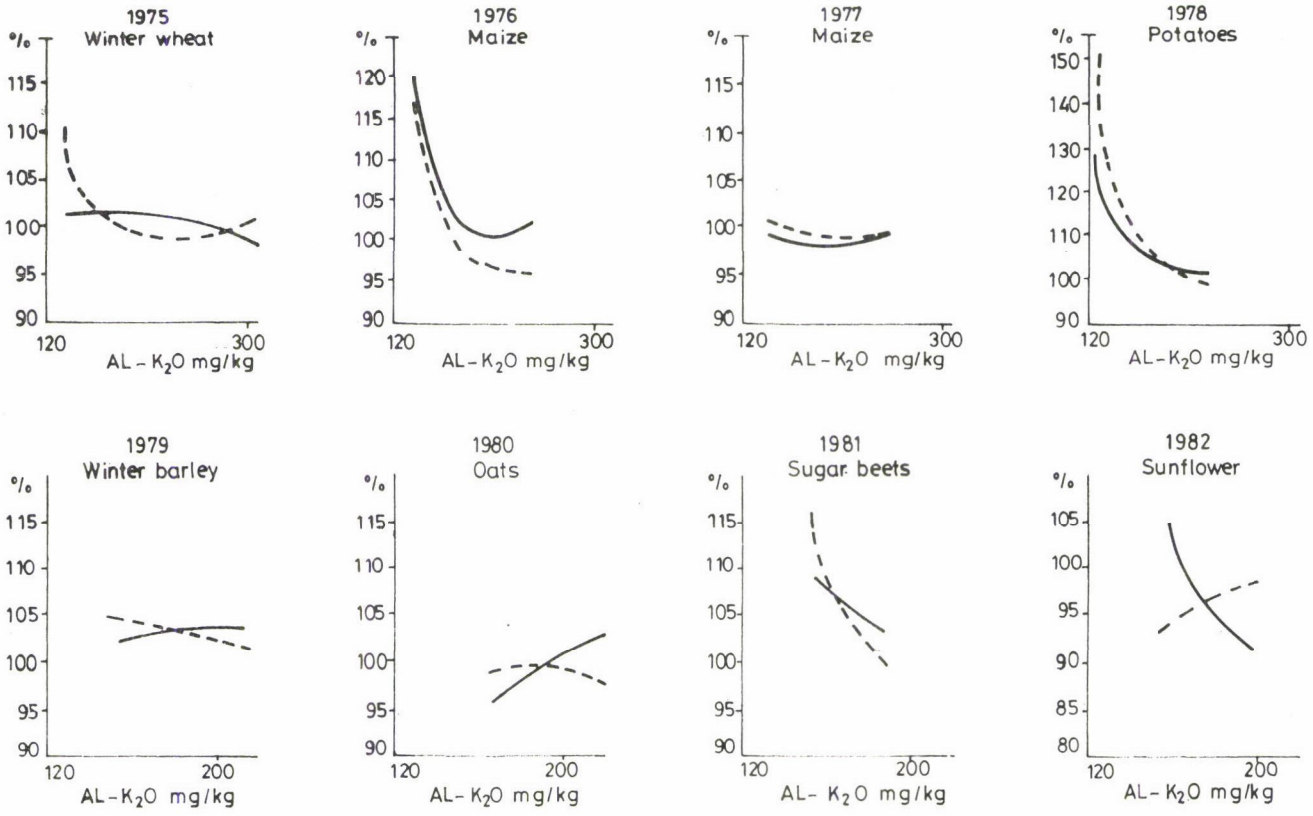


Fig. 4. Relationship between the AL-K_2O contents of the soil and the after-effect of the initial (building up) K-fertilization on the main yields


1975
Winter wheat
%
115
110
105
100
95
90
120
300
AL - K2O mg/kg
1976
Maize
%
120
115
110
105
100
95
90
120
300
AL - K2O mg/kg
1977
Maize
%
115
110
105
100
95
90
120
300
AL - K2O mg/kg
1978
Potatoes
%
150
140
130
120
110
100
90
120
300
AL - K2O mg/kg
1979
Winter barley
%
115
110
105
100
95
90
120
200
AL - K2O mg/kg
1980
Oats
%
115
110
105
100
95
90
120
200
AL - K2O mg/kg
1981
Sugar beets
%
115
110
105
100
95
90
120
200
AL - K2O mg/kg
1982
Sunflower
%
105
100
95
90
85
80
120
200
AL - K2O mg/kg

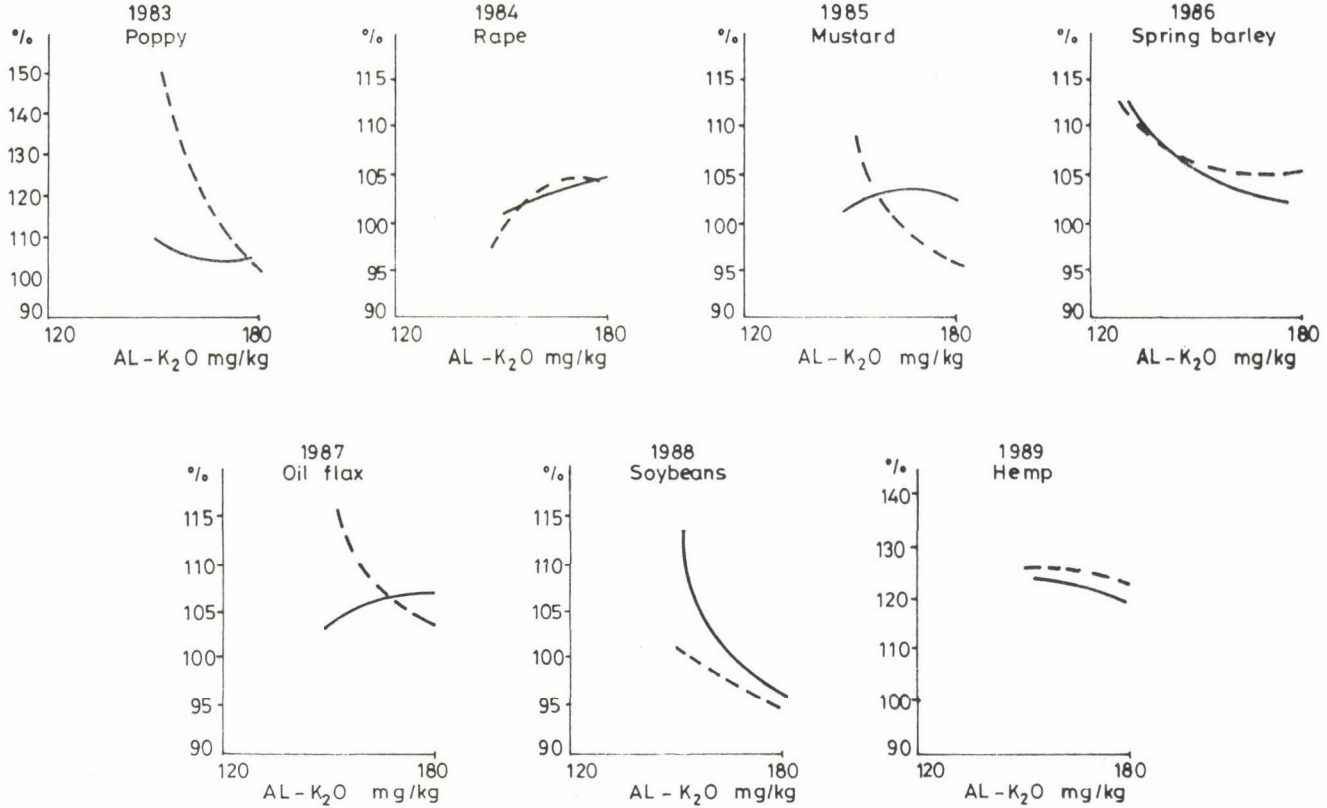


Fig. 5. Relationship between the AL-K_2O contents of the soil and the effect of the maintaining (fresh) K fertilization on the surplus in yield, % (yield on fresh K = 0 kg/ha = 100%) (——— 100; - - - - 200 kg/ha fresh K_2O/year)

ment). Initial quick high fixation of build-up doses was followed by an equilibrium state in AL-K contents from the 8th year of the trial. As an effect of 100 kg/ha maintaining yearly applications of K_2O, the extractable AL-K_2O content was increased by 24 mg/kg up to 1980, and by 76 mg/kg up to 1986. In the case of 200 kg/ha/year doses, the increases were 72, and 144 mg/kg, respectively. The fixation of the 500–1000–1500 kg/ha initial doses could not be counterbalanced at the beginning by the 100–200 kg/ha yearly applications, but later on, by the effects of positive K-balance saldos, after a while even in the plots initially receiving building up doses of K, AL-K contents began to increase.

(C) *Connection between the AL-K_2O content of soil and the yields*

The connection between the AL-K_2O contents of soils and the after-effects of the initial K-applications is shown in Fig. 4. On soils similar to that on which our experiment was set up, it is usually advisable to raise the AL-K_2O content up to 200–220 mg/kg. Higher doses are to be added to crops demanding larger amounts of K (potatoes, sugarbeet, maize, hemp, etc.), while K-fertilization of cereals and oilplants (except for poppy) can be moderated or even stopped. However, when the previous plant is alfalfa with its high K-uptake, normal K-fertilization of these plants is advisable.

The effectiveness of maintaining K-fertilization on different K-levels (i.e. on plots formerly fertilized with different initial [building-up] K-doses) is shown in Fig. 5. The effect or additive effect of fresh K-application is the highest — as also was the case with the after-effects — in hoed plants (potatoes, sugarbeets, maize, hemp). However, in the plots with previous application of K, the subsequent doses of fresh K resulted in lower and lower surpluses. As described by the Mitscherlich–Baule equation, the plants were able to take up high amounts of K given previously. Láng (1978) and Lásztity (1989) reported similar findings on a brown forest soil according to Ramann, and on a calcareous sandy soil, respectively. However, the after-effect of initial K-application decreased with time, and in the 16th year of the experiment, as the effect of fresh K-fertilization, the surplus in hemp stalks was significant even on plots previously fertilized with building-up doses of K (Table 6, Fig. 5).

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 319–332 (1991)

EFFECT OF AMENDMENTS ON GROWTH, NODULATION AND NITROGEN FIXATION BY BERSEEM (*TRIFOLIUM ALEXANDRINUM*) IN A CALCAREOUS SALINE-ALKALI SOIL

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(Received: 11 May, 1989; accepted: 17 October, 1989)

A pot experiment was conducted to study germination, growth and nodulation of Berseem in a saline-alkali soil treated with organic and inorganic amendments (alone and in conjunction) for reclamation. Higher salt concentration and exchangeable sodium were found to act additively to cause adverse effects on germination, growth and nodulation of berseem. The improvement in germination, growth and nodulation of berseem under various treatments was related to the extent of reclamation brought about. However, nitrogen content showed a reverse trend. The adverse effect of soluble salts and exchangeable sodium was more severe on nodulation as compared to that on plant growth. The nitrogen fixation efficiency of berseem was significantly improved by the use of organic or inorganic amendments, but a combination of both had an additive effect. Stepwise and multiple regression analyses were carried out for estimating and ranking the contribution of soil properties such as pH, EC and organic matter content towards germination, growth and nodulation of berseem.

Keywords: berseem, nitrogen fixation, nodulation, reclamation, saline-alkali soils

Introduction

Berseem is one of the favourite fodder crops. The importance of inoculating legume seeds with effective strains to obviate nitrogen deficiency and build up soil fertility is well-known. Saline and alkali soil constitute an unfavourable environment for growth and multiplication of rhizobia, root nodules and for the over-all growth of the plant. Several studies were carried out in the past to determine the salt tolerance of various legumes and rhizobial strains to the saline environment (Sen 1964, Pillai and Sen 1966, Subbarao et al. 1972, Bhardwaj 1974, Balasubramanian and Sinha 1976, Kumar and Garg 1981 and Kumar and Promila 1983) but there is lack of information as to how nodulation and growth are affected during reclamation. In the present investigation a calcarious saline-alkali soil in the process of reclamation under the influence of various organic and inorganic amendments was used to study germination, growth, nodulation and nitrogen fixation by berseem (*Trifolium alexandrinum*).

Materials and methods

This pot experiment was carried out on a typical calcareous saline-alkali soil of Rajasthan to study the effectiveness of some organic materials alone, and in conjunction with inorganic amendments in improving the saline-alkali soil and to evaluate their resultant effect on growth, nodulation and nitrogen fixation by berseem. The soil was first treated in the field and after leaching, was used for this experiment. Fifty percent of the gypsum requirement was met by applying gypsum (6400 kg/ha) or sulphur (1195 kg/ha). Four sources of organic materials, namely Dhaincha (*Sesbania aculeata*), farm yard manure (FYM), poultry manure and rice husk were used to supply 0.5% of organic carbon in the plough layer. A detailed description of each treatment is given below:

T_0 — Control: Leaching alone
T_1 — Gypsum: at the rate of 6400 kg/ha
T_2 — Sulphur: at the rate of 1195 kg/ha
T_3 — FYM: at the rate of 10,000 kg of organic carbon/ha
T_4 — FYM + gypsum: FYM at the rate of 10,000 kg of organic carbon/ha + gypsum at the rate of 6400 kg/ha
T_5 — FYM + sulphur: FYM at the rate of 10,000 kg of organic carbon/ha + sulphur at the rate of 1195 kg/ha
T_6 — Dhaincha (DA): at the rate of 10,000 kg of organic carbon/ha

Table 1

Physico-chemical characteristics of the soil profile used for the experiment

Characteristics	Horizon			
	A	B	C	D
Depth	0—15	15—30	30—45	45—70
Boundary	Diffuse	Diffuse	Clear	Clear
Colour	10 yr 7/1 light grey	10 yr 5/2 light grey	10 yr 4/1 dark grey	10 yr 3/2 very dark grey
Structure	Blocky	Blocky	Puddled	Puddled
Texture	Clay loam	Clay	Clay	Clay
Consistency	Compact	Compact	Sticky	Very sticky
pH	9.5	9.3	9.2	9.0
Permeability	5 lightly permeable	Less permeable	Impeded	Impeded
Moisture (% dry weight)	3.51	3.83	4.21	4.50
Organic C (%)	0.16	0.14	0.13	0.10
EC (m mhos/cm)	12.81	11.80	10.40	10.10
CEC (m e/100 g)	19.55	18.74	17.82	17.16
Mechanical analysis (percentage of mineral matter)				
Sand	55.5	50.6	46.2	44.5
Silt	12.5	12.9	13.4	14.2
Clay	32.3	36.5	40.4	41.3
Exch. Ca(m e/100 g)	4.2	4.6	4.2	3.7
Exch. Mg(m e/100 g)	8.1	8.1	7.8	7.8
Exch. K (m e/100 g)	0.3	0.2	0.2	0.2
Exch. Na(m e/100 g)	5.9	5.7	5.4	5.4
$CaCO_3$ (%)	4.2	3.9	5.1	6.8

T_7 — Dhaincha + gypsum: at the rate of 10,000 kg of organic carbon/ha + gypsum at the rate of 6400 kg/ha
T_8 — Dhaincha + sulphur: Dhaincha at the rate of 10,000 kg of organic carbon/ha + sulphur at the rate of 1195 kg/ha
T_9 — Poultry manure (PM): at the rate of 10,000 kg of organic carbon/ha
T_{10} — PM + gypsum: PM at the rate of 10,000 kg of organic carbon/ha + gypsum at the rate of 6400 kg/ha
T_{11} — PM + sulphur: PM at the rate of 10,000 kg of organic carbon/ha + sulphur at the rate of 1195 kg/ha
T_{12} — Rice husk (RH): at the rate of 10,000 kg of organic carbon/ha
T_{13} — RH + gypsum: RH at the rate of 10,000 kg of organic carbon/ha + gypsum at the rate of 6400 kg/ha
T_{14} — RH + sulphur: RH at the rate of 10,000 kg of organic carbon/ha + sulphur at the rate of 1195 kg/ha

The field was irrigated 15 times for the leaching of soluble salts. The details of soil profile of the area under study, also of irrigation water and chemical properties of the organic materials, have been described in Tables 1, 2 and 3. For the present experiment, soils were collected from these treatments after 250 days of incorporation followed by leaching. These soils were dried and sieved to pass through a 2 mm sieve, and then filled in pots of 5 kg capacity. Thirty berseem seeds were sown in each of the 60 pots (15 treatments × 4 replications) and irrigated by water containing 18 me/l of salts. This water was prepared by dissolving chloride salts of sodium and calcium in the ratio of 1 : 2. Germination was recorded after 10 days of sowing. Plants were thinned to 7 in all the pots after recording germination. These pots were irrigated

Table 2

The salinity and other characteristics of the original surface soil and the irrigation water used in reclamation of the area

Characteristics	Saturation extract of soil	Irrigation water
EC m mhos/cm	12.8	2.4
pH	9.4	8.1
Sodium adsorption ratio (SAR)	24.9	13.2
Na^+ (m e/l)	82.3	19.1
Ca^{++} (m e/l)	17.5	1.7
Mg^{++} (m e/l)	32.3	2.5
K^+ (m e/l)	0.5	0.3
CO_3^{2-} (m e/l)	0.7	0.3
HCO_3^{2-} (m e/l)	1.2	0.4
Cl^- (m e/l)	83.5	16.6
SO_4^{2-} (m e/l)	44.5	5.6
Boron (ppm)	0.1	0.1
Fluorine (ppm)	10.2	5.6
Water holding capacity (%)	29.50	
Gypsum requirement (kg/ha)	128.00	
Exchangeable Na (%)	30.23	
Hydraulic conductivity (cm/hr)	0.035	
Bulk density (g/cc)	1.84	
Dispersion coefficient (%)	79.05	
Aggregate size distribution:		
Aggregates > 0.25 mm (%)	6.85	
Aggregates 0.10—0.25 mm (%)	10.14	

Table 3

Chemical composition of organic materials

Constituents	Organic materials			
	Farm yard manure (FYM)	Dhaincha (DA)	Rice husk (RH)	Poultry manure (PM)
C %	32.91	48.32	42.33	34.51
N %	1.32	2.68	0.82	1.22
P %	0.63	0.39	0.17	0.44
S %	0.53	0.46	0.15	0.46
K %	0.53	0.86	2.88	0.78
C/N	25 : 1	14 : 1	52 : 1	28 : 1
C/P	52 : 1	124 : 1	249 : 1	78 : 1
C/S	62 : 1	101 : 1	282 : 1	75 : 1

Table 4

Effect of organic and inorganic amendments on the physical properties of the saline-alkali soil

Treatments	Hydraulic conductivity (cm/hr)	Bulk density (g/cc)	Water-holding capacity	Dispersion coefficient	Aggregate size > 0.25 mm	Distribution 0.10—0.25 mm
T_0	0.04	1.84	33.87 (31.05)	62.71 (78.98)	15.64 (7.26)	19.49 (11.13)
T_1	0.12	1.74	37.06 (36.31)	51.20 (60.73)	20.35 (12.09)	26.65 (20.11)
T_2	0.16	1.69	38.39 (38.55)	47.57 (54.45)	22.78 (14.99)	32.31 (28.57)
T_3	0.08	1.76	36.44 (35.29)	53.64 (72.91)	17.29 (8.83)	27.08 (20.73)
T_4	0.16	1.72	33.48 (38.71)	50.21 (59.05)	21.63 (13.82)	34.94 (32.80)
T_5	0.22	1.68	41.28 (43.51)	46.08 (47.40)	23.68 (16.13)	36.02 (34.59)
T_6	0.12	1.67	37.30 (36.71)	51.81 (35.73)	20.72 (12.52)	22.76 (14.97)
T_7	0.20	1.58	39.96 (41.25)	41.91 (44.61)	25.05 (17.93)	27.20 (20.90)
T_8	0.29	1.48	42.62 (45.85)	38.52 (51.49)	28.77 (23.16)	32.03 (28.13)
T_9	0.11	1.72	35.98 (34.51)	56.52 (69.55)	18.06 (9.61)	23.45 (15.64)
T_{10}	0.17	1.68	37.78 (37.53)	48.09 (53.39)	22.94 (15.19)	30.33 (25.50)
T_{11}	0.25	1.66	40.29 (41.81)	40.84 (42.80)	24.67 (17.42)	34.35 (31.83)
T_{12}	0.10	1.68	35.25 (33.31)	52.40 (62.77)	17.90 (9.45)	22.19 (14.26)
T_{13}	0.17	1.60	38.01 (37.91)	42.67 (37.75)	22.13 (14.19)	28.32 (22.36)
T_{14}	0.23	1.56	41.17 (43.33)	39.05 (39.69)	26.23 (19.53)	31.30 (26.99)
SEm ±	0.048	0.029	1.069	1.590	0.335	0.598
$LSD_{5\%}$	0.137	0.024	3.054	4.541	0.957	1.709

Note: Figures outside the parentheses are the transformed values of the percentage data following angular transformation, while figures within parentheses represent the re-transformed values (Fischer and Yates, 1963).

Table 5

Effect of organic and inorganic amendments on the salt balance and chemical characteristics of the saline-alkali soil

Treatments	pH	EC mmhos/cm	Organic carbon (%)	Exchangeable cations (percent of total CEC)			
				Na^+	Ca^{++}	Mg^{++}	K^+
T_0	9.30	12.55	2.40 (0.18)	31.17 (26.80)	30.31 (25.49)	36.90 (36.05)	15.52 (7.17)
T_1	8.85	10.88	2.90 (0.21)	26.46 (21.24)	34.10 (31.41)	36.25 (34.96)	15.94 (7.56)
T_2	8.80	10.05	2.90 (0.27)	26.22 (19.49)	35.31 (33.41)	36.56 (35.49)	16.47 (8.05)
T_3	9.20	12.15	3.22 (0.31)	29.94 (24.91)	30.99 (26.51)	36.82 (35.91)	16.07 (7.66)
T_4	8.82	10.10	3.52 (0.39)	25.97 (19.18)	35.29 (33.40)	36.84 (39.93)	15.74 (7.38)
T_5	8.70	8.15	3.75 (0.41)	24.85 (17.66)	36.47 (35.31)	36.71 (35.71)	15.68 (7.30)
T_6	8.85	10.40	3.01 (0.28)	27.54 (21.39)	34.21 (31.61)	36.03 (34.60)	16.25 (7.98)
T_7	8.70	9.80	3.14 (0.30)	23.84 (16.34)	37.36 (36.81)	36.25 (34.96)	16.80 (9.36)
T_8	8.40	6.75	3.38 (0.33)	21.77 (13.75)	39.16 (39.89)	35.96 (34.49)	17.17 (8.72)
T_9	9.20	11.80	3.34 (0.30)	29.95 (24.92)	31.69 (27.60)	36.47 (35.33)	15.86 (7.49)
T_{10}	8.75	10.55	3.43 (0.37)	25.39 (18.39)	35.90 (34.39)	36.26 (34.99)	16.21 (7.79)
T_{11}	8.60	7.90	3.71 (0.41)	23.36 (15.72)	37.39 (36.85)	36.05 (34.63)	16.75 (8.31)
T_{12}	9.15	11.65	3.12 (0.30)	29.83 (24.74)	32.03 (28.13)	36.65 (35.63)	16.65 (8.21)
T_{13}	8.80	9.90	3.33 (0.32)	26.36 (19.71)	34.83 (32.61)	36.59 (35.53)	16.31 (7.89)
T_{14}	8.65	7.25	3.52 (0.38)	23.76 (16.23)	36.83 (35.92)	36.54 (35.45)	16.91 (8.46)
SEm ±	0.07	0.54	0.07	0.89	0.86	Non-significant	0.08
$LSD_{5\%}$	0.20	1.54	0.19	2.55	2.46		0.23

Note: Figures outside parentheses are transformed values of the percentage data following angular transformation tables (Fischer and Yates, 1963) while figures within parentheses represent the re-transformed values.

at weekly intervals. The plants (aerial portion) were harvested at 50 days of growth after recording the plant height. The underground portion was carefully taken out from the pots, gently with all their root systems intact, with a stream of slowly running water to remove the adhering soil particles. In each pot nodules on every root were counted and the mean number of nodules per plant was calculated. The nodules were again sorted according to their size and location on main and secondary roots. The dry weight of the plant was determined by drying at 70 °C for 48 hours. The dry matter was then ground and the total N was determined by using a micro-kjeldahl method.

Results and discussion

The effect of incorporating amendments in the saline-alkali soil and their resultant effect on soil properties, plant growth, nodulation and N-fixation are as follows:

Effect on soil properties

The results of physical and chemical properties of saline-alkali soil as influenced by the use of organic materials and inorganic amendments followed by leaching, have been presented in Tables 4, 5 and 6. These data clearly reveal that organic materials or inorganic amendments when used alone, resulted in limited improvement, but they had an additive effect when used together. Sulphur, whether used alone or along with organic materials, proved its superiority over similar gypsum treatments. The ratio $Na^+/Ca^{2+} + Mg^{2+}$ de-

Table 6

Effect of organic and inorganic amendments on soluble cations and anions of the saline-alkali soil

Treatments	Anions (m e/100 g soil)					Cations (m e/100 g soil)			
	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	$\frac{SO_4^{2-}}{Cl^-}$	$Ca^{2+} + Mg^{2+}$	Na^+	K^+	$Na^+/Ca^{2+}+Mg^{2+}$
T_0	0.52	1.10	81.50	43.92	0.54	45.37	80.46	0.45	1.77
T_1	—	0.20	51.40	52.84	1.03	49.94	58.50	0.54	1.17
T_2	—	0.30	41.56	55.14	1.33	52.88	50.57	0.54	0.92
T_3	—	1.00	78.95	42.10	0.53	48.58	77.95	0.48	1.60
T_4	—	0.50	42.00	56.10	1.34	52.62	50.43	0.60	0.96
T_5	—	0.30	24.10	55.34	2.31	59.50	30.45	0.64	0.51
T_6	—	1.00	48.55	46.46	0.96	44.55	56.95	0.50	1.28
T_7	—	0.45	40.49	56.42	1.39	56.56	32.25	0.62	0.57
T_8	—	0.25	11.55	56.61	4.90	59.92	18.14	0.73	0.30
T_9	—	1.00	76.40	41.82	0.53	46.45	72.80	0.49	1.57
T_{10}	—	0.50	49.55	56.45	1.14	52.55	50.55	0.62	0.96
T_{11}	—	0.25	10.95	57.25	2.87	53.55	19.15	0.69	0.36
T_{12}	—	1.00	73.58	40.45	0.55	49.55	70.85	0.52	1.43
T_{13}	—	0.45	42.59	55.10	1.29	53.59	44.55	0.66	0.83
T_{14}	—	0.30	14.75	56.05	3.80	54.82	18.25	0.75	0.33

creased from 1.77 in control to 0.30 under treatment involving the addition of dha incha (*Sesbania aculeata* Pers.) + sulphur. The soil pH under various treatmentswas directly related with exchangeable sodium ($r = 0.98; p = 0.001$) and inversely with the content of exchangeable calcium ($r = 0.99$; $p = 0.001$). Improvement in pH and concentration of soluble salts was found to be associated with corresponding improvements in soil physical properties. Dhaincha, whether used alone or along with inorganic amendments (gypsum or sulphur), proved better in favourably improving the physical properties of the soil, salt balance, pH, EC, nutrient availability and crop yield over comparable treatments involving the organic materials (FYM, poultry manure or rice husk) because of its succulence, fast-decomposing nature and high content of calcium besides the production of sulphurous acid during its microbial transformation in the soil (Somani and Saxena 1981).

Effect on growth, nodulation and N-fixation

The data on germination, growth and nodulation presented in Table 7, show an increasing germination with reclamation. Improvements in germination were limited (although significantly higher over control) when organic materials and inorganic amendments were used alone, as compared to when

Table 7

Germination, growth and nodulation of berseem

Treatments	Germination %	Height cm	Number of nodules per plant	Dry weight of nodules per plant (mg)	Effective nodules %	Dry matter			N fixed mg/pot
						Per pot (gm)	% N	Total N	
T_0	50.3	11.6	24.7	22.9	18.1	2.6	3.48	9.05	39
T_1	72.3	18.1	34.7	30.7	38.6	4.7	3.25	15.28	121
T_2	76.7	21.3	41.7	36.7	49.3	5.2	3.23	16.80	155
T_3	70.7	15.3	29.7	27.3	30.9	3.8	3.37	12.81	82
T_4	77.7	27.9	45.3	39.9	64.6	7.1	3.22	22.86	167
T_5	87.7	31.1	60.7	52.6	76.5	7.8	3.19	24.88	231
T_6	73.3	15.9	34.3	30.4	39.8	4.3	3.27	14.06	121
T_7	80.7	30.8	51.3	44.6	72.1	7.2	3.21	25.36	202
T_8	89.3	35.1	65.7	55.2	85.5	8.7	3.13	27.23	293
T_9	69.3	13.9	28.3	26.1	29.1	4.1	3.39	13.90	109
T_{10}	75.7	26.1	42.3	37.1	59.9	6.4	3.23	20.67	168
T_{11}	83.3	27.8	53.7	46.2	69.9	7.2	3.17	22.82	245
T_{12}	66.3	13.1	25.7	23.5	26.6	3.2	3.33	10.66	98
T_{13}	76.3	24.1	41.7	36.7	54.5	6.1	3.21	19.58	165
T_{14}	86.7	27.2	52.3	45.3	66.0	7.1	3.18	22.58	225
SEm ±	2.134	0.591	2.076	2.231	1.808	0.256	—	—	4.85
$LSD_{5\%}$	6.095	1.688	5.929	6.372	5.164	0.731	—	—	13.85

they were used in conjunction. For instance the germination of 59.3% in control increased to 72.3%, 76.7%, 73.3% 80.7% and 89.3% with gypsum, sulphur, dhaincha, dhaincha + gypsum and dhaincha + sulphur, respectively. Of all the organic materials, dhaincha proved the best. The better effect of sulphur as compared to that of gypsum could be attributed to greater improvement in salt balance, soil physical and chemical properties (Tables 4, 5 and 6). The improvements brought about when organic materials and inorganic amendments were used together are related to their additive ameliorative effect as is evident from the data presented in Tables 4, 5, 6 and 7. Within the pH and EC range of this study, the improvement in germination, height, number of nodules per plant, dry weight of nodules and effective nodulation was from 50.3 to 89.3%, 11.6 to 35.1 cms, 24.7 to 65.7, 22.9 to 55.2 mg and 18.1 to 85.5%, respectively. These data thus clearly reveal that the adverse effect of salts and exchangeable sodium was much more severe on nodulation, as compared to crop growth. Bajpai et al. (1974) also observed that the inhibitory level of soluble salts and alkalinity was much lower for nodulation than that for crop growth. These growth improvements could partly be related to the increase in the content of soil organic matter. The organic amendments would also provide nutrition for plants and the microorganisms, resulting in the formation of more numerous effective nodules, as also reported by Sirry et al. (1980).

It is interesting to point out here that it was only up to 10.05 millimhos/cm of salts and an exchangeable sodium percent of 21.14 (pH 8.66) that large pinkish nodules, having effective symbiotic properties, were observed. Above these limits, the effective nodulation decreased progressively, and mostly small greenish nodules, scattered on secondary roots, were observed. Survival and growth of rhizobia, even up to pH 10, has also been observed by Lakshmi-Kumari et al. (1974) and Bhardwaj (1975). Salinity and/or alkalinity has been shown to reduce the production of pink pigment and enhance greening (Kumar and Garg 1981, Kumar and Promila 1983). The effect of low levels was mainly on the reduced development of the pigment whereas that of the higher levels was both on the development as well as greening, which began earlier and was also enhanced. The factors which affect the stability of the pigment are largely unknown, however, the process being that of oxidation (Kumar and Promila 1983). The increased greening may be expected with increasing salinity which leads the plant metabolism to a more oxidative side (Dey and Tilak 1984). All the improvements in germination, growth and nodulation under the ameliorative effect of various organic materials and inorganic amendments are related to the extent of reclamation produced. This is evident from highly significant values of the coefficient of correlations between soil physical and chemical properties, and crop growth and nodulation presented in Table 8.

Table 8

Value of coefficient of correlation (r) for relationship between soil character and germination, growth and nodulation of berseem

Relationship between	pH	EC	Organic C	Exchangeable		Soluble SO_4^{2-}/Cl^-	$\frac{Na^+}{Ca^{2+}+Mg^{2+}}$	Dispersion coefficient	Structural Index	Hydraulic conductivity
				Na^+	Ca^{2+}					
Germination	—0.89**	—0.90**	0.74**	—0.91**	0.89**	0.77**	0.91**	—0.71**	0.89**	0.93**
Height	—0.91**	—0.86**	0.68**	—0.95**	0.94**	0.76**	—0.93**	—0.59*	0.91**	0.92**
Number of nodules	—0.92**	—0.93**	0.66**	—0.94**	0.94**	0.86**	—0.95**	—0.62*	0.94**	0.96**
Dry weight of nodules	—0.91**	—0.93**	0.67**	—0.94**	0.93**	0.84**	—0.95**	—0.61*	0.93**	0.95**
Effective nodules	—0.93**	—0.89**	0.71**	—0.96**	0.96**	0.78**	—0.95**	—0.65**	0.93**	0.95**
Dry matter	—0.91**	—0.87**	0.70**	—0.95**	0.94**	0.76**	—0.93**	—0.62*	0.92**	0.93**
Percent nitrogen	0.96**	0.88**	—0.63**	0.95**	—0.94**	—0.73**	0.93**	0.79**	—0.90**	—0.91**
Total nitrogen	—0.88**	—0.81**	0.67**	—0.92**	0.92**	0.70**	—0.89**	—0.58*	0.88**	0.90**
Nitrogen fixed	—0.94**	—0.96**	0.69**	—0.96**	0.96**	0.88**	—0.96**	—0.67**	0.96**	—0.99**

* Significant at 5 %
** Significant at 1 %

The reduction in the exchangeable sodium, leaching of toxic salts and improvement in soil physical properties, possibly affected the efficiency of *Rhizobium* in the soil. In addition, the direct effect of sodium on the nutrition of the micro-organisms and the plants was minimized after the reclamation (Somani and Saxena 1981, 1982). Moreover, water stress is known to affect the formation and longevity of leguminous root nodules, so also nitrogen fixation (Sprent 1971). The decrease of osmotically induced water stress with reclamation, in part, was also responsible for better nodulation, growth and N-fixation. A slight desiccation, due to high salinity, leads to a strong inhibition of respiratory activity and N-fixation. No reversal of these effects is found and the nodules are probably shed by the plant (Lie 1974).

The percent nitrogen content in the dry matter, however, decreased with reclamation under different treatments as compared to control (Table 7). This could be attributed to the dilution effect of enhanced plant growth due to lowered salt stress and exhangeable sodium content. Stunting of plant growth with increasing osmotic stress leads to accumulation of nitrogen (Somani 1980). Despite the decreased percentage of N in the dry matter, with progressive reclamation the total amount of N-fixed improved (Table 7). Wilson (1970), Sprent (1972) and Kumar and Garg (1981) also observed that N-fixing efficiency of nodules was reduced by salinization of 9.0 mmhos/cm and above. The reduced dry matter yield relates to the reduced leaf area, which restricts the production of photosynthesis. As the process of nitrogen fixation by the nodules also requires the supply of photosynthesis from the leaves, the reduced supply of photosynthesis may thus in part be responsible for the reduced amount of nitrogen fixed by the nodules under saline-alkali conditions. The SO_4^{2-} : Cl^- ratio also increased from 0.54 in control to as high as 4.90 (Table 6) with progressive reclamation. A highly significant correlation for the relationship between SO_4^{2-} : Cl^- ratio and plant growth, nodulation and N-fixation (Table 8) indicate the antagonistic effect of higher contents of Cl^- in the growth medium. Kumar and Promila (1983) also observed SO_4^{2-} salinity to be less harmful than Cl^- salinity.

Multiple and stepwise regression analysis studies

In studies based on simple correlations it is not possible to separate the direct effect of particular soil property from the indirect effect caused by its own relationship with another soil character, unless multiple regression is employed. Therefore, multiple regression equations were worked out and are presented in Table 9. From these equations it appears that the accumulation of excessive salts suppressed the initiation of nodules but not their further development. This becomes evident from a comparison between the number of nodules per plant and dry weight of nodules per plant. In fact, the decreased

Table 9

Multiple regression equations for predicting germination, growth and nodulation of berseem from properties of saline-alkali soil

Dependent variables	Constant B	Independent variables			R^2
		pH(X_1)	EC(X_2)	Organic C(X_3)	
Germination	240.83	—18.66*	—1.42	45.04*	0.89***
Height	223.85	—24.01**	0.22	28.74	0.86***
Number of nodules	251.24	—20.53	—3.46*	22.79	0.91***
Dry weight of nodules	197.03	—15.75	—2.77	22.33	0.90***
Effective nodules	576.31	—61.95***	0.236	83.42*	0.92***
Dry matter	53.81	—5.73*	0.02	7.92*	0.88***
Percent nitrogen	0.02	0.39***	—0.01	—0.25	0.96***
Total nitrogen	187.23	—20.96**	0.82	26.63*	0.84***
Nitrogen fixed	1286.14	—112.47**	—18.27**	168.07*	0.95***

* Significant at 5%
** Significant at 1%
*** Significant at 0.1%

number of nodules per plant with increasing salinity was responsible for the low weight of dry nodules per plant, showing thereby that the dry weight per nodule was not affected by salinity. Balasubramanian and Sinha (1976) also reported higher dry weight per nodule of the salt stressed plants. While high exchangeable sodium as reflected through soil pH increased the percent of nitrogen in the dry matter, it had a detrimental effect on germination, height, effective nodulation, dry matter production, total nitrogen recovered through dry matter and nitrogen fixed through nodules. Evidently, an increase in nitrogen percentage in dry matter with increasing pH was due to poor plant growth. Organic matter made a significant contribution towards germination, effective nodulation, dry matter production and nitrogen fixation. In addition to its role in improving soil physical properties, organic materials ensured a sustained supply of micronutrients essential for symbiosis.

Sophisticated variable selective regression models which make it possible to rank and estimate the significance of the contribution of various soil characters that affect germination growth and nodulation of berseem are now available. Draper and Smith (1966) and Cholitkul and Tyner (1971) considered stepwise regression to be the best of the variable selection regression. The stepwise regression analysis as presented in Table 10 clearly reveals that electrical conductivity largely affected germination, number of nodules per plant, dry weight of nodules per plant and nitrogen fixed symbiotically; whereas exchangeable sodium mainly governed the height of the plant, effective nodulation, dry matter per pot, percent of nitrogen content in the dry matter and total nitrogen recovered through dry matter. Although organic matter contri-

Table 10

Stepwise regression analysis of germination, growth, nodulation of berseem with properties of saline-alkali soil

Variables		R	R^2	ΔR^2	Overall F***
Dependent	Independent				
Germination	EC	0.89797	0.80635	0.80635	54.13
	Organic C	0.92451	0.85471	0.04836	35.29
	pH	0.94359	0.89037	0.03566	29.78
Height	pH	0.90642	0.62160	0.82160	59.86
	Organic C	0.92988	0.86467	0.04307	38.33
	EC	0.93006	0.86501	0.00034	23.49
Number of nodules per plant	EC	0.93780	0.87947	0.87947	94.85
	pH	0.94999	0.90248	0.02302	55.52
	Organic C	0.95454	0.91114	0.00866	37.59
Dry weight of nodules per plant	EC	0.93171	0.86809	0.86809	85.55
	pH	0.94241	0.88814	0.02005	47.64
	Organic C	0.94916	0.90090	0.01276	33.33
Effective nodules	pH	0.93243	0.86942	0.86942	86.55
	Organic C	0.97061	0.92277	0.05335	71.69
	EC	0.96064	0.92283	0.00005	43.85
Dry matter per pot	pH	0.90927	0.82678	0.82678	62.05
	Organic C	0.93869	0.88114	0.05437	44.48
	EC	0.93872	0.88114	0.00004	27.19
Percent nitrogen	pH	0.96580	0.93278	0.93278	180.35
	Organic C	0.97356	0.94782	0.01500	108.93
	EC	0.97564	0.95187	0.00406	72.52
Total nitrogen	pH	0.88362	0.78078	0.78078	46.30
	Organic C	0.91227	0.83223	0.05145	29.76
	EC	0.91705	0.84098	0.00875	19.39
Nitrogen fixed	EC	0.95933	0.92031	0.92031	150.12
	pH	0.97104	0.94291	0.02261	99.10
	Organic C	0.97930	0.95903	0.01612	85.83

*** All significant at 0.1%

buted significantly to germination, according to the growth and nodulation of berseem as revealed in stepwise regression analysis, the contribution came at a second or third stage.

It is interesting to point out that the salts did not significantly affect germination and dry weight of nodules per plant as revealed through multiple regression equations. It ranked first in the stepwise regression analysis. This could be attributed to two factors; first, in multiple regression equations a considerably large contribution is accounted for causes beyond the ones also under study, and secondly, in the stepwise regression analysis the indirect effect of any factor to its relationship with another factor is not separated. However, the data in Table 10 clearly reveal that the pH, EC and organic mat-

ter content of saline-alkali soils had a significant bearing on both germination growth and nodulation of berseem.

In sum and substance, it can be concluded that both organic and inorganic amendments brought marked improvements in salt balance of the soil which in turn had stimulatory effects upon the growth and nodulation of berseem. A combination of organic and inorganic amendments provided better results, while sulphur + dhaincha yielded the best results.

Acknowledgement

Thanks are due to Prof. S. N. Saxena, Head, Department of Soil Science and Agriculture Chemistry, RCA, Udaipur for providing necessary facilities during the course of this investigation.

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 333–337 (1991)

INFLUENCE OF SALT STRESS ON PHOTOSYNTHESIS IN HORSEGRAM (*DOLICHOS BIFLORUS* L.) AND CHICKPEA (*CICER ARIETINUM* L.)

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(Received: 3 September, 1990; accepted: 18 October, 1990)

Horsegram and chickpea are the leguminous crops which possess typical C_3 features. An attempt was made to determine whether salt stress causes any shifts in the basic C_3 nature of these crops. Sodium chloride salinity caused very minor alterations in the values of carbon isotope ratios in leaves of both these legumes. The analysis of early photosynthetic products revealed that largest proportion of ^{14}C was incorporated in the C_3 metabolites in the salt-stressed plants. Salinity also caused stimulation of activity of a key photorespiratory enzyme glycolate oxidase. These observations indicate that the salinity does not cause any C_3–C_4 shift in either horsegram or chickpea.

Keywords: *Dolichos biflorus* L., horsegram, *Cicer arietinum*, chickpea, photosynthesis, salinity

Introduction

In recent years considerable attention has been paid to the mechanism of C_4 photosynthetic pathway due to the fact that C_4 plants have several ecological adaptive features (Jenkis and Hatch 1982). There are few reports which indicate that plants adapt to conditions of soil salinity (Physiological drought) by altering their mode of photosynthesis. Thus both C_3-C_4 and C_3-CAM shifts have been reported in some plant species (Winter and Willert 1972, Shomer-Ilan and Waisel 1973, Ferron et al. 1978). In the present investigation an attempt has been made to study the influence of sodium chloride salinity on the mode of photosynthesis in two legume species of horsegram and chickpea.

Materials and methods

For salt tolerance studies, a sand culture technique was used. Ten healthy seeds of local and chafa cultivars of horsegram and chickpea respectively were sown in polyethylene culture containers (42 cm diameter with a hole at the bottom) containing acid free silica sand of about 100 mesh, in the month of October. Hoagland nutrient solution (1/2 strength) was added to these plants and this nutrient dose was repeated every 6th day throughout crop duration. After one month of establishment, the plants were subjected to treatment of sodium chloride. O (control), and 50 mM levels of NaCl were selected. The salt treatment was given along with culture medium three times weekly, alternating with equal amounts of water to avoid evaporation and excess salt accumulation. The fresh leaf material from identical posi-

tions of three-month-old plants was selected for the photosynthetic studies. The short term (10 sec) photosynthetic ^{14}C product analysis studies were performed according to the method standardized in our laboratory (Bhivare, Nimbalkar and Chavan 1988). The carbon isotope ratios from the oven-dried leaf material of both the plants was estimated by following the method of Smith and Epstein (1971). The activity of a key photorespiratory enzyme glycolate oxidase was estimated according to the method of Hess and Tolbert (1967).

Results and discussion

The effect of NaCl salinity on ^{13}C values and the distribution of radioactivity in ethanol soluble fraction following 10 sec light $^{14}CO_2$ assimilation in the leaves of horsegram and chickpea is depicted in Table 1. The ratio of

Table 1

Effect of NaCl salinity on $\delta^{13}C$ values (0/00) and the distribution of radioactivity (% of total radioactivity counted on chromatogram) in ethanol soluble fraction following 10 sec light $^{14}CO_2$ assimilation in the leaves of Horsegram and Chickpea

δ ^{13}C value (0/00)	Horsegram		Chickpea	
	NaCl treatment (mM)			
	Control	50 mM	Control	50 mM
	—26.44	—26.59	—29.00	—28.20
Compound				
PEP	0.47	0.69	6.77	5.32
PGA	Trace	0.93	3.19	6.38
SDP	Trace	7.53	—	—
SMP	3.56	1.39	2.71	Trace
Unidentified sugar phosphate	4.03	1.51	—	—
Phosphorylated compounds	8.06	12.05	12.67	11.70
Aspartate	10.12	14.37	2.44	2.02
Glycine-serine	3.62	1.04	1.06	13.28
Glutamate	15.86	23.87	5.45	5.65
Alanine	19.33	20.97	53.15	38.41
Methionine	3.81	2.32	—	—
Amino acids	52.74	62.57	62.10	59.36
Glycerate	2.23	2.20	4.22	5.65
Glycolate	0.85	1.04	—	—
Malate	6.14	3.24	4.37	7.70
Succinate	1.41	0.81	3.25	5.06
Citrate	1.22	Trace	4.45	10.43
Organic acids	11.85	7.29	16.29	28.84
Sucrose	21.14	13.34	8.94	Trace
Glucose	0.97	1.51	Trace	Trace
Fructose	5.14	3.24	Trace	Trace
Sugars	27.25	18.09	8.94	Trace

Values are mean of three determinations.

C^{12} to C^{13} in organic carbon is one of the recently discovered characteristics distinguishing C_3 and C_4 species. This isotope composition is conventionally expressed as ^{13}C, the difference in per mil of the $^{13}C/^{12}C$ ratio of the sample relative to a standard ^{13}C value ranges from — 10.0 to — 18.0 0/00 for C_4 species compared to — 24.00 to — 30.00 0/00 for C_3 species. It is clear from the data that leaves of both the plants exhibit ^{13}C values in the range typical for most of C_3 species. There is only a slight change in ^{13}C values under saline conditions which indicates that there is very little change in the basic nature of the carboxylation process in both these legumes. Few attempts have been made to study the relationship between salinity and carbon isotope fractionation ratio (Neales et al. 1983, Downton et al. 1985, Seemann and Critchley 1985). These workers have suggested that salinity induces the shift in ^{13}C values, not by affecting a change in the mode of carboxylation, but by affecting the degree of limitation of CO_2 assimilation by the diffusion process. According to them, such a shift results from stomatal closure. These workers have also attributed this shift to reduced Ci (internal CO_2 concentration) due to diffusional limitations with increasing salt stress.

It is also clear from the table that, during a period of 10 seconds, ^{14}C has entered in phosphorylated compounds, amino acids, and organic acids, as well as sugars in the leaves of control plants of both the species. The results obtained in the present investigation clearly indicate the operation of C_3 pathway in both the plants, because C_3 compounds (alanine, PEP, PGA, glycine serine and glycerate) receive more radioactivity than the C_4 compounds (malate and aspartate). The percentages of radioactivity in C_3 and C_4 compounds under control and saline conditions in horsegram and chickpea are 25.65, 25.83, 68.39, 69.04 and 16.26, 17.61, 6.81 and 9.72, respectively. It appears that alanine receives maximum radioactivity in both of the plant species. Alanine is found to be one of the major recipients of label in initial phase of carbon assimilation (Huber et al. 1973, Kennedy and Laetsch 1974 and Raghavendra and Das 1977). The formation of alanine in C_3 plants takes place much more quickly and early, as compared to that in C_4 ones, because alanine is much nearer to PGA, than to OAA in C_4 plants. Thus the occurrence of heavy labelling in alanine in horsegram and chickpea can be justified through its synthesis from 3 PGA. Besides alanine, another C_3 compound which has received considerable label is glycerate. The formation of glycerate through dephosphorylation reaction from PGA is well established in many plants (Belan 1976a, b). The photorespiratory intermediates such as glycolate and glycine-serine also get labelled during the early phase of photosynthesis in C_3 species due to predominance of photorespiration, which occurs simultaneously with photosynthesis (Tolbert 1971, Edwards and Walker 1983). The C_4 acids, namely aspartate and malate, also account for about 16.26% and 6.81% of the total label during short term photosynthesis in horsegram and

chickpea, respectively. The label in citric acid, succinic acid and glutamic acid clearly suggests that photosynthetically fixed ^{14}C may be immediately metabolized in TCA cycle (Ferron et al. 1978).

The incorporation of ^{14}C in sucrose during short term photosynthesis in both the control plants suggests that the operation of the Calvin-cycle is quite a rapid process. It can be seen from the table that due to salt stress there are several changes in the pattern of ^{14}C fixation. The labelling in sugars is lowered due to salt stress and this might be due either to the slowing down of the Calvin-cycle, or the diversion of carbon to other products. This is supported by the increase in labelling of both PGA and PEP under saline conditions in horsegram and only of PGA in chickpea. Among TCA cycle intermediates, the incorporation of radioactivity in C_3 compounds is slightly increased over that of control due to salt stress.

A number of attempts have been made to discover a basic change in the carboxylation pattern induced by salinity. Shomer-Ilan and Waisel (1973) have detected PGA in salt-depleted and aspartate in salt-treated Aleuropus litoralis. Ferron et al. (1978) investigated the effect of salinity on the carboxylation pathways in halphyte (*Plantago maritima*, var. graminacea) and in a glycophyte (*Plantago lanceolata* L.). They analysed the photosynthetic products during 1 min period of photosynthesis with respect to C_4 compounds (malate + aspartate), photorespiratory C_3 compounds (glycine + serine) and respiratory compounds (Glutamate). They also measured the ratio of label in C_3 compounds (PGA, glycerate, PEP, glycine-serine and alanine) to label in C_4 compounds (malate + aspartate). They observed that under saline conditions the photosynthetic carbon metabolism in a glycophyte (*P. lanceolata*) showed strong catabolic disturbances while in halophytic *P. maritima* a distinct shift from C_3 to C_4 pathway was noticed. In the present investigation, although the salt stress causes changes in the pattern of labelling of short term photosynthetic products, the alterations do not indicate any shift in the labelling from C_3 compounds to C_4 compounds. Some of these alterations in the allocation of label to various intermediates may be regarded as "catabolic disturbances", as reported by Ferron et al. (1978). The ^{13}C values also support this observation.

The activity of enzyme glycolate oxidase which catalyzes the oxidation of glycolate to glyoxylate during photorespiratory process is regarded by many workers as an indicator of photorespiratory rate (Zelitch 1979). Our observations revealed that NaCl salinity caused a stimulation of activity of this enzyme in both the leguminous plants. In horsegram the increase (over control) in enzyme activity in the leaves of salt-treated plants was 175%, while it was 25% in chickpea. Since photorespiration is a component of C_3 photosynthesis, these observations also support the view that salinity does not cause any C_3-C_4 shift in either horsegram or chickpea.

Acknowledgements

One of the authors (CVM) is thankful to CSIR, New-Delhi for financial assistance. Our gratitude is further extended to Prof. B. N. Smith (USA) for his help in ^{13}C analysis.

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 339–345 (1991)

PHYSIOLOGICAL ANALYSIS OF POPULATION DENSITY EFFECT ON RAPE (*BRASSICA CAMPESTRIS* L.) I. GROWTH ANALYSIS

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(Received: 24 March, 1990; accepted: 23 January, 1991)

An experiment was conducted to study the pattern of dry matter production and growth of three rape cultivars under four population densities. Total dry matter and leaf area index (LAI) increased significantly with the increasing population density and the highest values for all the cultivars were recorded under the highest population density. Total dry matter increased as plant age advanced, but LAI attained its peak value at certain days after sowing, and thereafter declined. Among the growth attributes, crop growth rate (CGR) at both pre- and post-flowering stages, net assimilation rate (NAR) and leaf weight ratio (LWR) at pre-flowering stage and relative growth rate (RGR) and specific leaf area (SLA) at post-flowering stage were significantly affected by population density. Significant differences among the cultivars for total dry matter and all the growth attributes, except pre-flowering RGR, were also observed. Simple correlation coefficients indicated that seed yield had significant positive correlation with post-flowering LAI and NAR at most of the population densities.

Keywords: rape, dry matter, leaf area, relative growth rate, net assimilation rate, pre-flowering, post-flowering

Introduction

Rape has an important position among those crops that yield vegetable oil. But rape seed production is inadequate compared to its demand. Thus, the agronomists and plant scientists in various parts of the world have been trying to improve the rape seed yield. The causes for low yields in rape are many. Of them, proper cultural practices particularly the plant population density or row spacing is most important and has a profound effect on plant morphology by changing crop environments. Much work has been done on the effect of population density, row spacing or seeding rate on the yield of rape, but the physiological analysis of rape in relation to population density is almost nil.

Dry matter accumulations and yields vary with population density and this variation may be associated with the physiological responses such as leaf area, RGR, NAR, LAR, SLA, LWR and CGR (Mendham et al. 1981, Buttery 1969, Clark and Simpson 1978, Islam and Paul 1986).

The objective of the present investigation was to study the effect of four population densities, mediated by four row spacings, on growth attributes of three rape cultivars. A subsequent paper deals with the effect of population density on seed yield and its components.

Materials and methods

The experiment was conducted at the experimental field of the Department of Botany, University of Rajshahi, Bangladesh. The three cultivars of rape (*Brassica campestris* L.) used were Sampad, Tori 7 and TS-72.

The field was well ploughed and all weeds eradicated. A basal dose of fertilizers with the ratio of urea: muriate of potash: triple super phosphate (2:1:1) was applied in the field. The experiment was arranged in a randomized complete block design with three replications. The four spacings used were 15 cm, 30 cm, 45 cm and 60 cm between the rows. In each spacing there were 14 rows of 8 m length directed north to south. Four experimental rows were allocated for each cultivar for sampling and the two borders were considered as non-experimental. Seeds of the three cultivars were sown on 23rd October, 1987.

Fourteen days after sowing (DAS), seedlings were thinned to a plant-to-plant spacing of 10 cm, in order to make the plant population density 66.7 plants m^{-2} (667,000 plants ha^{-1}), 33.3 plants m^{-2} (333,000 plants ha^{-1}), 22.2 plants m^{-2} (222,000 plants ha^{-1}) and 16.7 plants m^{-2} (167,000 plants ha^{-1}) in 15 cm, 30 cm, 45 cm and 60 cm spacings, respectively.

The experiment included 8 harvests, the first of which was done at 18 DAS, and the subsequent harvests were at 7 days intervals. At the time of harvest, the plants were cut down at the cotyledon node and were separated into leaves, stem + other parts and pods, if present. All plant parts were separately dried in an oven at about 80 °C for 24^h and then weighed. The leaf area was measured by the disc method (Nangju and Wanki 1980). From the dry weights of different plant parts and leaf area data, various growth attributes were calculated following the classical technique of growth analysis (Radford 1967). The analysis of variance was done according to randomized complete block design, and LSD values were calculated. Simple correlation coefficients among the growth attributes and that with seed yield were calculated.

Results and discussion

Total dry matter and LAI were significantly affected by population density throughout the growth stages. Total dry matter and LAI increased with the increase of population density and the highest values were recorded under the highest population density (Figs 1 and 2). Total dry matter increased continuously until the final sampling date, whereas LAI increased up to a certain limit and its maximum attainment was recorded at 39 DAS in all the spacings in both Sampad and TS-72 and at 32 DAS in all the spacings except 30 cm in Tori 7, then declined with the advancement of plant age. The spacing order was 15 cm, 30 cm, 45 cm, 60 cm for these two characters. Throughout the growing period the lowest population density had the highest number of branches, as well as number of leaves per plant, but the lower leaf area per unit ground area. Otherwise, increased plant densities adversely affected plant growth, but not the total dry matter production per unit area, because the quantitative reduction in plant growth was probably compensated by the increase in plant population, as well as leaf area per unit ground area.

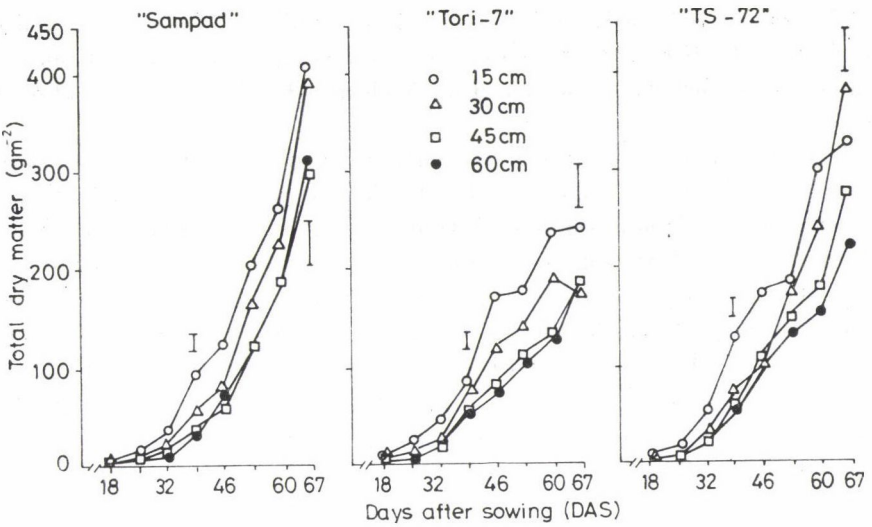

Fig. 1. Influence of population density on total dry matter (TDM) of three rape cultivars at successive growth stages

The increase in plant density caused an increase in LAI, which was responsible for much light interception and, as a result, higher total dry matter production in closely spaced plants was observed. Similar results were also reported by Watson (1947), Buttery (1969) and Enyi (1973). The higher dry matter in narrow spacing was also observed by Patil and De (1978) in rape.

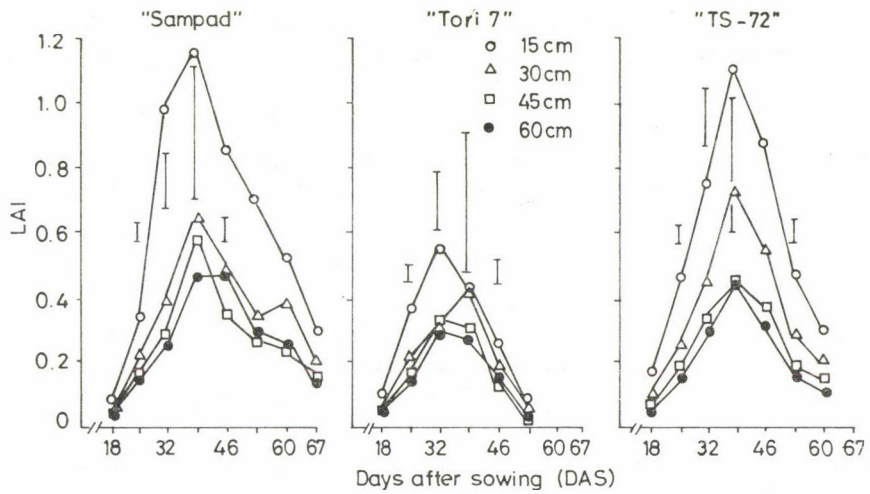

Fig. 2. Influence of population density on leaf area index (LAI) of three rape cultivars at successive growth stages

The analysis of variance indicated that population density had a significant effect on CGR at both pre- and post-flowering stages, on RGR and SLA only at the post-flowering stage, and on NAR and LWR only at pre-flowering stage.

The CGR increased consistently with increasing plant densities at both pre- and post-flowering stages (Table 1). Ahlawat and Saraf (1983) observed similar results in pigeon pea. Population density had a significant effect on RGR only at post-flowering stage, where RGR decreased with the increasing of population density (Table 1). This adverse relationship of RGR with plant population may probably be due to the interplant competition. Similar results were also reported by Enyi (1973) in soybean. At the pre-flowering stage, NAR increased with the increase of population density (Table 1), probably because of the higher LAI in the higher population densities.

Population density had no significant effect on LAR at both stages; but the decline of LAR in higher plant densities (Table 1) at the pre-flowering stage may be due to the differential distribution of photosynthesis between leaf growth and other plant organs. Ahlawat and Saraf (1983) in pigeon pea reported similar results. Higher SLA values at both stages were recorded under higher population densities except in 45 cm spacing at the post-flowering stage, whereas the highest LWR values at both stages were recorded under the lower population densities (Table 1).

The simple correlation analysis revealed that seed yield was positively associated with both post-flowering LAI and NAR in most of the population densities (Table 2). A positive correlation of seed yield with LAI was also reported by Clarke and Simpson (1978) in *B. napus* and Chaturvedi et al. (1988) in *B. juncea*. A significant positive correlation of seed yield with post-flowering RGR (only in 45 cm spacing) was supported by Thurling (1974). The seed yield was also correlated positively with post-flowering LAR (in 30 cm spacing), with CGR at both stages in 60 cm spacing, with post-flowering LAR in 30 cm spacing, and with pre-flowering RGR in 45 cm spacing. The seed yield showed a significant negative correlation with pre-flowering CGR in 30 cm spacing, with pre-flowering RGR in 45 cm spacing, and with both pre-flowering CGR and LAR in 15 cm spacing. CGR at both stages was positively correlated with pre-flowering LAI in most of the spacings, but pre-flowering CGR had significant negative correlation with post-flowering LAI (in 30 and 45 cm spacings). A significant negative association of CGR at both stages with post-flowering RGR was recorded in some of the spacings. CGR at both stages had a significant positive association with pre-flowering NAR in 15 cm spacing, and had a significant negative association with pre-flowering LAR in most of the spacings. Post-flowering RGR correlated positively with pre-flowering LAR (in 15 cm spacing) and negatively with pre-flowering NAR (in 15 and 45 cm spacings). NAR

Table 1

Mean growth attributes of three rape cultivars as influenced by population density

Cultivar	Pre-flowering				Post-flowering			
	Sampad	Tori 7	TS-72	Mean	Sampad	Tori 7	TS-72	Mean
Spacing (cm)								
				CGR ($g\ m^{-2}\ day^{-1}$)				
15	4.29	2.14	1.62	2.68	10.56	6.21	7.50	8.09
30	2.52	1.26	0.72	1.50	11.96	3.45	8.62	8.01
45	1.72	0.90	0.62	1.08	9.39	4.21	6.49	6.70
60	1.55	0.56	0.76	0.96	9.86	4.22	5.23	6.44
Mean	2.52	1.22	0.93		10.44	4.52	6.96	
$LSD_1 = 0.33$, $LSD_2 = 0.38$, $LSD_3 = 0.66$					$LSD_1 = 0.65$, $LSD_2 = 0.77$, $LSD_3 = 1.33$			
				RGR ($g\ g^{-1}\ day^{-1}$)				
15	0.16	0.18	0.14	0.16	0.05	0.06	0.07	0.06
30	0.13	0.18	0.12	0.14	0.07	0.06	0.09	0.07
45	0.14	0.16	0.14	0.15	0.07	0.11	0.09	0.09
60	0.14	0.12	0.18	0.15	0.08	0.08	0.08	0.08
Mean	0.14	0.16	0.14		0.07	0.08	0.08	
LSD_1 = NS, LSD_2 = NS, $LSD_3 = 0.03$					$LSD_1 = 0.006$, $LSD_2 = 0.007$, $LSD_3 = 0.01$			
				NAR ($g\ cm^{-2}\ day^{-1}$) X 10^{-4}				
15	7.19	11.60	5.38	8.06	10.02	18.42	14.71	14.38
30	6.41	10.05	4.33	6.93	16.55	16.67	24.70	19.16
45	6.71	8.88	5.10	6.90	36.38	12.68	15.20	21.42
60	6.74	5.68	7.66	6.69	36.16	13.46	15.38	21.67
Mean	6.76	9.05	5.62		24.78	15.35	17.50	
$LSD_1 = 0.66$, $LSD_2 = 0.76$, $LSD_3 = 1.32$					$LSD_1 = 6.13$, LSD_2 = NS, $LSD_3 = 12.27$			
				LAR ($cm^2\ g^{-1}$)				
15	222.24	161.76	256.14	139.30	32.49	100.53	75.79	69.60
30	181.13	175.02	283.29	213.15	34.18	84.68	82.18	67.01
45	208.49	183.31	287.10	226.30	38.67	102.76	74.26	71.90
60	207.95	206.46	236.99	217.13	38.63	103.06	73.26	71.65
Mean	204.95	181.64	265.88		35.99	97.76	76.37	
$LSD_1 = 17.38$, LSD_2 = NS, $LSD_3 = 34.76$					$LSD_1 = 3.96$, LSD_2 = NS, $LSD_3 = 7.92$			
				SLA ($cm^2\ g^{-1}$)				
15	286.38	273.96	347.30	302.55	269.09	464.76	603.17	445.67
30	286.03	279.35	345.07	303.48	191.96	371.44	473.82	345.74
45	283.35	284.31	332.83	300.16	242.01	341.68	337.80	307.16
60	277.65	287.36	314.48	293.16	283.18	348.92	310.91	314.17
Mean	283.35	281.24	334.92		246.54	381.72	431.30	
$LSD_1 = 33.97$, LSD_2 = NS, $LSD_3 = 67.96$					$LSD_1 = 55.98$, $LSD_2 = 64.65$, $LSD_3 = 111.24$			

Table 1 (cont'd)

Cultivar	Pre-flowering				Post-flowering			
	Sampad	Tori 7	TS-72	Mean	Sampad	Tori 7	TS-72	Mean
Spacing (cm)								
				LWR ($g\ g^{-1}$)				
15	0.744	0.653	0.741	0.713	0.135	0.137	0.176	0.149
30	0.627	0.645	0.767	0.680	0.134	0.194	0.183	0.170
45	0.717	0.658	0.865	0.747	0.110	0.254	0.189	0.185
60	0.737	0.744	0.761	0.747	0.093	0.243	0.202	0.180
Mean	0.706	0.675	0.783		0.118	0.207	0.187	
$LSD_1 = 0.03$, $LSD_2 = 0.03$, $LSD_3 = 0.06$					$LSD_1 = 0.03$, $LSD_2 = NS$, $LSD_3 = 0.06$			

LSD_1 for difference between cultivar means, LSD_2 for difference between spacing means and LSD_3 for difference between spacing means within a cultivar. All LSD values at 5% level of significance. NS = Non-significant.

at both stages had a significant positive correlation with post-flowering LAR in 30 cm spacing. The pre-flowering NAR correlated negatively with post-flowering LAI (in 45 cm spacing) which corroborated the findings of Thurling (1974).

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Table 2

Simple correlation coefficients between growth attributes and seed yield in four spacings

	Spacing (cm)					Spacing (cm)			
	15	30	45	60		15	30	45	60
1×2	0.73*	0.41	0.73*	0.25	4×6	0.09	−0.46	0.96**	−0.13
1×3	0.96**	0.87**	0.89**	0.90**	4×7	−0.05	0.65	−0.68*	0.04
1×4	−0.23	−0.91**	−0.98**	0.88**	4×8	0.56	0.95**	0.70*	0.12
1×5	0.55	0.30	0.15	0.17	4×9	0.18	0.67*	0.54	−0.07
1×6	−0.97**	0.05	−0.92**	−0.19	4×10	−0.55	0.84**	−0.65	0.15
1×7	0.81**	−0.57	0.63	0.40	4×11	0.88**	0.97**	0.46	0.82**
1×8	−0.59	−0.75*	−0.70*	0.09	5×6	−0.51	0.88**	−0.51	0.85**
1×9	−0.90**	−0.89**	−0.66	−0.45	5×7	0.13	0.01	0.32	−0.34
1×10	0.51	−0.88**	0.57	0.14	5×8	−0.27	−0.63	−0.45	−0.18
1×11	−0.63	−0.89**	−0.37	0.83	5×9	−0.36	0.14	0.43	0.30
2×3	0.77*	0.79*	0.85**	0.41	5×10	0.09	−0.12	0.08	−0.34
2×4	−0.44	−0.02	−0.60	−0.06	5×11	−0.55	−0.58	−0.95**	0.41
2×5	0.26	−0.55	−0.54	−0.72*	6×7	−0.90**	−0.18	−0.74*	−0.29
2×6	−0.79*	−0.85**	−0.41	−0.62	6×8	0.49	−0.52	0.82**	−0.53
2×7	0.81**	−0.01	0.19	0.47	6×9	0.96**	0.39	0.32	0.33
2×8	−0.63	0.22	−0.25	0.17	6×10	−0.44	−0.12	−0.49	−0.46
2×9	−0.93**	−0.71*	−0.98**	−0.81**	6×11	0.53	−0.47	0.69*	0.09
2×10	0.58	−0.17	0.38	0.24	7×8	−0.52	0.75*	−0.92**	−0.19
2×11	−0.78*	−0.02	−0.37	0.83**	7×9	−0.97**	0.54	−0.14	−0.46
3×4	−0.04	−0.59	−0.87**	0.81**	7×10	0.53	0.72*	0.03	0.44
3×5	0.54	−0.15	−0.22	−0.10	7×11	−0.50	0.64	0.60	−0.05
3×6	−0.97**	−0.44	−0.70*	−0.50	8×9	0.53	0.49	0.14	−0.35
3×7	0.87**	−0.41	0.48	0.39	8×10	−0.98**	0.76*	0.06	0.20
3×8	−0.39	−0.36	−0.42	0.43	8×11	0.83**	0.95**	0.72*	0.04
3×9	−0.94**	−0.97**	−0.85**	−0.54	9×10	−0.49	0.78*	−0.41	−0.06
3×10	0.32	−0.72*	0.69*	0.36	9×11	0.62	0.65	−0.45	−0.25
3×11	−0.48	−0.57	0.02	0.63	10×11	−0.73*	0.79*	−0.04	−0.08
4×5	−0.45	−0.59	−0.27	0.28					

*, ** indicate significant at 5% and 1% levels, respectively.

1 = Pre-flowering CGR, 2 = Post-flowering CGR, 3 = Pre-flowreing LAI, 4 = Post-flowering LAI, 5 = Pre-flowering RGR, 6 = Post-flowering RGR, 7 = Pre-flowering NAR, 8 = Post-flowering NAR, 9 = Pre-flowering LAR, 10 = Post-flowering LAR and 11 = Seed yield.

Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 347–353 (1991)

PHYSIOLOGICAL ANALYSIS OF POPULATION DENSITY EFFECT ON RAPE (*BRASSICA CAMPESTRIS* L.) II. YIELD AND YIELD COMPONENTS

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(Received: 24 March, 1990; accepted: 17 May, 1990)

Seed yield and its components of three rape cultivars grown at four population densities were evaluated during the growing season of 1987. Among the plant characters related to flowering, days taken to first flowering and days taken to end of flowering were earlier in the higher densities. Plant height and leaf number at first flowering decreased with the increase in population density. Among the yield components, plant height at harvest, number of branches per plant, number of pods per plant, number of seeds per pod, total weight per plant and harvest index decreased with the increase in population density. Seed yield per unit area increased with the increase in population density and the highest value (2023 $kg \cdot ha^{-1}$) was recorded under the highest population density. Seed yield was positively correlated with plant height at harvest, number of branches per plant, number of seeds per pod and total weight per plant in most of the population densities and with total number of pods per plant and 1000-seed weight in a few population densities.

Keywords: population density, flowering, pod number, seed yield, correlation coefficient

Introduction

In rape and mustard, important morphological characters like number of branches per plant, number of pods per plant and number of seeds per pod are greatly influenced by population density. Numbers of pods per plant and seeds per pod were reduced by higher seeding rate (Clark and Simpson 1978) but increased seeding rates and higher population density resulted in a higher number of pods per unit ground area (Degenhardt and Kondra 1981, Mendham et al. 1981).

The aim of this investigation was to study the effect of four population densities on seed yield and its components of three rape cultivars.

Materials and methods

Seeds of three rape (*Brassica campestris* L.) cultivars (Sampad, Tori 7 and TS-72) were sown on 23rd October, 1987 in the experimental field of the Department of Botany, University of Rajshahi, Bangladesh. Details of field preparation, plot arrangement, plot size and management of the trial are given in the preceeding paper (Roy and Paul 1991).

Days taken to first flowering, days taken to end of flowering and leaf number and plant height at first flowering date were recorded. At the time of final harvest, the following characters were measured: plant height (cm), number of primary branches, total number of pods per plant, number of seeds per pod, seed-husk weight ratio, total weight per plant (g), 1000-seed weight (g), harvest index and seed yield per unit area ($kg \cdot ha^{-1}$).

An analysis of variance was done according to the randomized complete block design, and LSD values at 5% level were calculated. Simple correlation coefficients between characters were calculated.

Results and discussion

All the characters related to flowering responded significantly to the population density. The flowering was earlier in both 15 and 45 cm spacings. The end of flowering was earlier and hence the duration of flowering was shorter in the high population density treatment (Table 1). The plant height and leaf number were decreased with the increase in population density (Table 1). The greater interplant competition for soil nutrients and incident light in the high population density treatment was probably responsible for progressive reduction in plant size, branch and leaf numbers. Due to the reduction in branch number, both the end of flowering and duration of flowering were hastened. The higher plant population apparently resulted in more soil moisture use in the vegetative phase, leaving less moisture for utilization at the flowering and seed ripening stages. This might be another cause of the shortening of duration of flowering in higher population densities. Similar findings were reported by Degenhardt and Kondra (1981) and Patil and De (1978) in rape, Kumar and Tripathi (1975) and Shastry and Kumar (1981) in rai.

Among the yield components, plant height at harvest, number of branches per plant, total number of pods per plant, number of seeds per pod, and total plant weight were significantly affected by population density. Those characters decreased with the increase in population density (Table 2). The increased interplant competition for soil moisture, nutrients and incident solar radiation that normally attended high population density was probably responsible for the progressive reduction in plant size as well as in the number of leaves and branches, characters that have been shown to be essential for optimum reproductive development. For this reason the total number of pods per plant decreased with the increase in population density. Singh et al. (1978) and Clark and Simpson (1978) reported similar results.

In contrast to the higher population, an improvement in the above yield attributes at lower plant density could be attributed to the availability of relatively more space per plant which, in turn, assisted the individual plants to utilize the available resources with the least competition. Similar observations were reported by Mudhokar and Ahlawat (1979) and Singh and Singh (1984) in rape. The 1000-seed weight was unaffected by population

Table 1

Plant characters related to flowering of three rape cultivars as affected by population density

Cultivar	Spacing (cm)				Mean
	15	30	45	60	
	Days taken to first flowering (DAS)				
Sampad	40.73	40.70	37.33	37.77	39.02
Tori 7	20.33	21.33	20.80	21.33	20.95
TS-72	22.27	25.20	24.57	25.10	24.28
Mean	27.78	29.08	27.57	28.07	
$LSD_1 = 0.34$, $LSD_2 = 0.39$, $LSD_3 = 0.68$					
	Days taken to end of flowering (DAS)				
Sampad	56.90	54.30	57.47	56.23	56.22
Tori 7	33.50	34.73	35.70	33.77	34.42
TS-72	38.77	43.73	43.83	44.27	42.65
Mean	43.05	44.25	45.67	44.76	
$LSD_1 = 0.75$, $LSD_2 = 0.87$, $LSD_3 = 1.50$					
	Duration of flowering (days)				
Sampad	16.17	13.60	20.13	18.47	17.09
Tori 7	13.17	13.40	14.90	12.43	13.47
TS-72	16.50	18.53	19.30	19.17	18.37
Mean	15.25	15.18	18.11	16.69	
$LSD_1 = 0.34$, $LSD_2 = 0.40$, $LSD_3 = 0.69$					
	Plant height at first flowering (cm)				
Sampad	29.90	29.60	38.07	41.73	34.83
Tori 7	13.17	14.53	17.30	19.07	16.02
TS-72	17.60	19.03	19.43	23.57	19.91
Mean	20.22	21.05	24.93	28.12	
$LSD_1 = 0.84$, $LSD_2 = 0.97$, $LSD_3 = 1.68$					
	Number of leaves at first flowering				
Sampad	6.40	7.40	8.00	8.77	7.64
Tori 7	4.90	5.50	5.73	7.00	5.78
TS-72	4.37	5.47	5.80	6.40	5.51
Mean	5.22	6.12	6.51	7.39	
$LSD_1 = 0.32$, $LSD_2 = 0.37$, $LSD_3 = 0.64$					

LSD_1 for difference between cultivar means, LSD_2 for difference between spacing means and LSD_3 for difference between spacing means within a cultivar.

density, corroborating the work of Degenhardt and Kondra (1981) and Kondra (1975).

The per hectare seed yield was higher in the higher population density and the highest yield for all the cultivars was recorded in the highest popula-

Table 2

Seed yield and its components of three rape cultivars as affected by population density

Cultivar	Spacing (cm)				Mean
	15	30	45	60	
	Plant height at harvest (cm)				
Sampad	91.33	106.00	110.66	114.66	105.66
Tori 7	46.00	57.33	62.66	64.00	57.50
TS-72	70.66	84.00	91.33	104.00	87.50
Mean	69.11	82.44	88.22	94.22	
$LSD_1 = 2.64$, $LSD_2 = 3.05$, $LSD_3 = 5.29$					
	Number branches per plant				
Sampad	6.50	7.80	9.16	11.66	8.78
Tori 7	2.66	2.83	4.10	5.20	3.70
TS-72	4.93	5.30	6.06	6.40	5.67
Mean	4.70	5.31	6.44	7.75	
$LSD_1 = 0.53$, $LSD_2 = 0.61$, $LSD_3 = 1.06$					
	Number of pods per plant				
Sampad	79.00	96.00	138.67	186.33	125.00
Tori 7	62.00	99.00	141.67	148.67	112.83
TS-72	88.33	150.33	177.33	190.00	151.50
Mean	76.44	115.11	152.56	175.00	
$LSD_1 = 13.15$, $LSD_2 = 15.19$, $LSD_3 = 26.30$					
	Number of seeds per pod				
Sampad	19.97	21.13	21.30	26.73	22.28
Tori 7	12.96	13.26	14.43	15.56	14.05
TS-72	15.93	16.16	17.00	21.50	17.65
Mean	16.29	16.85	17.58	21.26	
$LSD_1 = 1.09$, $LSD_2 = 1.26$, $LSD_3 = 2.19$					
	Seed-husk weight ratio				
Sampad	1.08	1.25	1.35	1.22	1.22
Tori 7	1.04	1.31	1.33	1.49	1.29
TS-72	1.36	1.41	1.18	1.55	1.83
Mean	1.16	1.32	1.29	1.42	
$LSD_1 = NS$, $LSD_2 = NS$, $LSD_3 = 0.38$					
	Total weight per plant (g)				
Sampad	13.73	18.83	24.10	31.60	21.97
Tori 7	4.90	7.40	11.03	14.03	9.34
TS-72	8.50	16.50	18.70	21.23	16.23
Mean	9.04	14.24	17.94	22.29	
$LSD_1 = 2.26$, $LSD_2 = 2.61$, $LSD_3 = 4.54$					

Table 2 (cont'd)

Cultivar	Spacing (cm)				Mean
	15	30	45	60	
	Harvest index (%)				
Sampad	28.57	32.43	33.63	33.47	32.02
Tori 7	37.60	38.67	41.07	45.73	40.77
TS-72	38.67	36.87	38.13	40.40	38.52
Mean	34.95	35.99	37.61	39.87	
$LSD_1 = 3.32$, $LSD_2 = NS$, $LSD_3 = 6.64$					
	1000-seed weight (g)				
Sampad	3.30	3.32	3.59	3.05	3.25
Tori 7	2.79	2.53	2.65	3.20	2.79
TS-72	2.96	2.67	2.79	3.05	2.87
Mean	3.02	2.81	3.01	3.10	
$LSD_1 = 0.33$, $LSD_2 = NS$, $LSD_3 = 0.66$					
	Seed yield per hectare (kg)				
Sampad	2624	2020	1754	1765	2041
Tori 7	1267	955	999	1086	1077
TS-72	2179	2020	1576	1434	1801
Mean	2023	1665	1443	1427	
$LSD_1 = 260$, $LSD_2 = 301$, $LSD_3 = 521$					

LSD_1 for difference between cultivar means, LSD_2 for difference between spacing means and LSD_3 for difference between spacing means within a cultivar. All LSD values at 5% level of significance. NS = Non-significant.

tion density (Table 2). In the lower population density, per hectare seed yield was considerably reduced primarily because of the decrease in plant stand per unit area, which failed to compensate for the increased number of plants per unit area at higher population density. Similar results were reported by Singh et al. (1963) and Maini et al. (1964) in rape.

Simple correlation coefficients indicated that seed yield had a significant positive correlation with plant height at harvest, number of primary branches and total weight per plant in all the spacings (Table 3). The seed yield was also associated positively with total number of pods per plant in 30 and 60 cm spacings, with number of seeds per pod in all but 45 cm spacing, and with 1000-seed weight in 15 cm spacing. A similar positive correlation was also recorded by Clark and Simpson (1978) and Thurling (1974) in *B. napus*, Paul et al. (1976) in mustard and Joarder et al. (1978) in rape. A significant negative correlation of seed yield with harvest index was observed only in 45 cm spacing. The total number of pods per plant was positively correlated with plant height (in 60 cm spacing). The number of seeds per pod had a significant positive correlation with the number of primary branches in all

Table 3

Simple correlation coefficients between seed yield and its components in four spacings

	Spacing (cm)					Spacing (cm)			
	15	30	45	60		15	30	45	60
1×2	0.94**	0.97**	0.91 **	0.77*	3×7	0.09	0.08	0.26	−0.54
1×3	0.58	0.01	−0.03	0.88**	3×8	0.52	0.29	0.10	0.43
1×4	0.92**	0.88**	0.85**	0.86**	3×9	0.68*	0.40	0.31	0.69*
1×5	0.09	−0.16	0.09	−0.28	4×5	0.01	−0.03	0.04	−0.28
1×6	0.54	0.55	0.71*	−0.49	4×6	0.54	0.29	0.69*	−0.42
1×7	−0.55	−0.66	−0.64	−0.78*	4×7	−0.58	−0.49	−0.71*	−0.69*
1×8	0.83**	0.93**	0.84**	0.89**	4×8	0.89**	0.78*	0.73*	0.91**
1×9	0.73*	0.88**	0.90**	0.84**	4×9	0.76*	0.73*	0.63	0.89**
2×3	0.58	0.02	−0.13	0.47	5×6	0.33	−0.19	−0.28	−0.01
2×4	0.97**	0.89**	0.87**	0.83**	5×7	0.76*	0.63	0.46	−0.45
2×5	0.06	−0.11	−0.19	−0.32	5×8	0.12	−0.11	−0.36	−0.39
2×6	0.56	0.51	0.82**	−0.31	5×9	0.45	0.09	−0.12	−0.06
2×7	−0.55	−0.45	−0.86**	−0.87**	6×7	−0.16	−0.56	−0.74*	0.19
2×8	0.81**	0.89**	0.94**	0.96**	6×8	0.81**	0.52	0.71*	−0.37
2×9	0.71*	0.82**	0.87**	0.77*	6×9	0.84**	0.39	−0.47	0.54
3×4	0.51	−0.16	−0.41	0.69*	7×8	−0.47	0.56	−0.88**	−0.82**
3×5	0.56	0.34	0.19	0.09	7×9	−0.13	−0.42	−0.68*	−0.55
3×6	0.41	−0.11	−0.37	−0.43	8×9	0.93**	0.96**	0.93**	0.95**

*, ** indicate significant at 5% and 1% levels, respectively.

1 = Plant height at harvest, 2 = Number of primary branches, 3 = Total number of pods per plant, 4 = Number of seeds per pod, 5 = Seed-husk weight ratio, 6 = 1000-seed weight, 7 = Harvest index, 8 = Total weight per plant and 9 = Seed yield.

the spacings, with 1000-seed weight in 45 spacing and total weight per plant in all the spacings. The 1000-seed weight was positively correlated with the number of seeds per pod in 45 cm spacing and with total weight per plant in 15 and 45 cm spacings. A significant positive correlation with the number of seeds per pod and number of primary branches in all the spacings was supported by Clark and Simpson (1978) in *B. napus* and by Ahmed (1980) in rape.

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 355–358 (1991)

Plant protection

INVESTIGATIONS ON THE POPULATION DENSITY OF *MELOIDOGYNE HAPLA* CHITWOOD ON GRAPEVINE (*VITIS VINIFERA* AND *VITIS VINIFERA* × *SEYVE-VILLARD*) HYBRIDS

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(Received: 5 February, 1990; accepted: 13 April, 1990)

Considerable differences were established in the susceptibility and resistance to *Meloidogyne hapla* in the case of 34 hybrids of *Vitis vinifera* and *Vitis vinifera* × *Seyve-Villard*. The investigated vineyard is on sandy soil in the Danube–Tisza Mid-region, in Hungary. The highest population density was 1005 J_2/250 g of soil of the rhizosphere of cv. Pinot noir, with high galls and egg-masses indices. The lowest population density was found in the rizosphere of the hybrid KM-151, 20 J_2/250 g of soil, with no galls or egg-masses. Susceptible hybrids, the most susceptible (e.g. Pinot noir, Chardonnay, Kecskemét 7) and least susceptible hybrids (e.g. Favorit, Bikavér 17, Kecskemét 13) and one resistant hybrid (KM-151) are all hybrids of *Vitis vinifera*. There are differences in susceptibility among the interspecific hybrids, too. The susceptibility and tolerance of *Vitis vinifera* hybrids to *M. hapla* were studied for the first time on this occasion.

Keywords: Nematode, *Meloidogyne hapla*, variety of vine, *Vitis vinifera*, resistance, tolerance

Introduction

Some species of the genus *Meloidogyne* are known as pests on grapevines. These species could cause weakness, such as vines that do not grow enough to permit training onto a trellis, or are unsuccessful for replanting of vineyards (Faulkner and McElroy 1964, Goodey et al. 1965, Esser et al. 1968, Katalan–Gateva and Choleva–Abdzhieva 1977, Raski 1988, Scotto La Massése et al. 1988). Since the chemical control against *Meloidogyne* species has some difficulties and drawbacks, the study of the susceptibility or resistance of different varieties has great significance. Former authors have studied the resistance of rootstock varieties (Bouquet and Dalmasso 1976, Dalmasso and Cuani 1976, Bouquet et al 1982, Stirling and Cirami 1984, Santo and Hackney 1986). We had the opportunity to survey the population density of *M. hapla* on the root and in the rizosphere of 34 hybrids, primarily of *Vitis vinifera*, as well as some interspecific hybrids of *Vitis vinifera* × *Seyve-Villard* 12375 and 18315. It is important to mention that on the sandy soil of the Danube–Tisza Mid-Region the grapevine has been cultivated on ungrafted propagative materials for a long time, over an area of about 100,000 acres.

Materials and methods

Soil and root samples were collected from the rizosphere of 34 hybrids of grapevine on sandy soil in the Danube–Tisza Mid-Region in the vineyard of Experimental Station Miklóstelep of Research Institute for Viticulture and Enology (Kecskemét) between June and October in three successive years, 1986, 1987 and 1988. Ten soil samples were taken from each row. The vineyard was planted with cuttings. The plants were placed in 3.5×1.2 ms.

Two hundred and fifty grams of soil from each soil sample were processed by the sieve-decanting technique for extracting second stage juveniles (J_2). Four replicates of 1 ml from standard nematode suspension (20 ml) were counted and the average was multiplied by 20 ml for estimation the total number of J_2/250 g of soil.

Root samples were investigated for the presence of galls and egg-masses according to the Taylor and Sasser (1978) scale. Egg/egg-mass rates were calculated by dissolving one egg-mass in an aqueous solution of sodium hypochlorite (commercial bleach) according to the Hussey and Barker (1973) technique. An average of 4 replicates was taken from each sample.

Results and discussion

Most of the grapevine hybrids in Experimental Station Miklóstelep were very highly susceptible to Northern root-knot Nematode, *M. hapla*; gall and egg-mass indices were 4–5, 5–5, with very high population density of the second stage juveniles (J_2) up to 1005 J_2/250 g of soil, with a very high reproduction rate of eggs, 1353 eggs/egg-mass.

One variety, KM-151, was apparently resistant: neither galls nor egg-masses were found on the roots, with a very low population density 20 J_2/250 g of soil. Two hybrids, Boglárka and Kecskemét 13, were slightly susceptible: 1–2 galls and egg-masses, with low reproduction rate of eggs 270–330 eggs/egg-mass.

The highest population density, 1005, 770, 734, 680, J_2/250 g of soil and high reproduction rate of eggs 989, 693, 650, 1353 eggs/egg-mass appeared on hybrids planted in rows Nos 31, 32, 20 and 34 respectively. This high population density may be the main reason for dead plants in rows Nos 28, 31 and 34. The varieties planted in rows Nos 32 and 20 exhibited good growth condition, and may tolerate these levels of infestation. In the meantime, for the variety in row No. 28, only two weak plants were still alive with a low population density 15 J_2/250 g soil that had a medium reproduction rate of eggs 315 eggs/egg-mass and galls and egg-mass index 3–2 (Table 1).

Our findings of J_2 density are higher than the findings of Katalan–Gatevy and Choleva–Abdzihieva (1976) 22–240 J_2 in 200 g of soil in Bulgarian vineyards.

Root symptoms appear as small to medium swellings or galls on the tips of feeder roots as well as on small roots, each swelling or gall containing 7–10 second stage juveniles (J_2), and more than one female was found in galled roots. White egg-masses with females and sometimes males, were concentrated on small and larger roots, with/without gall formation.

Table 1

Juveniles, eggs/egg-mass number as well as gall and egg-mass indices on grapevine (Vitis vinifera and Vitis vinifera × Seyve-Villard) hybrids
(Kecskemét-Miklóstelep, Hungary, 1986—1988)

Row+ Nos	Grapevine cultivars	No. of J_2/250 g of soil++	No. of eggs/egg-mass+++	GI/EI++++
31	Pinot noir	1005	989	5—5
32	Chardonnay	770	693	5—5
20	Kecskemét 7	734	650	5—5
34	Musc. Hambourg	680	1353	3—5
22	Kármin	480	325	5—5
26	Jubileum 75	480	425	5—5
15	Göcseji zamatos	420	256	3—3
12	Zefir	415	500	3—5
4	Zweigelt	405	330	5—5
27	Cs 6	405	946	3—5
14	Lakhegyi mézes	320	700	5—5
16	Médea	275	400	4—5
10	Pintes	275	359	3—5
21	Bianca	260	650	2—4
17	Cegléd szépe K—73	215	450	4—5
2	Chasselas b. Fr. 38—95	180	650	5—5
33	Boglárka	165	270	1—2
6	Bikavér 17	165	400	3—3
8	Bikavér 12	135	700	3—2
24	Karát	135	373	3—3
23	Kurucvér	110	650	5—5
29	Kecskemét 13	95	330	1—2
37	Red Veltliner	85	470	2—2
9	Király leányka 21	80	400	4—3
38	Chasselas doré	70	733	3—2
3	Pölöskei muskotály	70	500	3—5
5	TF. Oporto	65	430	3—3
19	Favorit	60	600	2—2
1	Chasselas r. Fr. 36—28	60	406	5—5
7	Bikavér 17	45	280	2—2
13	Zenit	40	950	5—5
11	Zengő	25	810	4—5
18	KM 151	20	0.0	0—0
28	Furmint T/92	15	315	3—2
±	LSD at 5%	±4.62	±212.8	

+ Row number indicates the number of the row of each variety: In rows Nos 25, 30, 35, 36 the plants died, so were not included in the survey. In rows Nos 28, 31, 34 one or two plants remained alive.

++ Average of three replicates.

+++ Average of four egg-masses counts.

++++ GI = Gall index; EI = eggmass index: no galls or egg-masses = 0; 1—2 galls or egg-masses = 1; 3—10 = 2; 11—30 = 3; 31—100 = 4; more than 100 = 5 (Taylor and Sasser, 1978).

Susceptible and resistant, as well as the most susceptible (e.g. Pinot noir, Chardonnay, Kecskemét 7) and least susceptible hybrids (e.g. Favorit, Bikavér 17, Kecskemét 13) could be found among the *Vitis vinifera* hybrids and there was a great difference in susceptibility among the interspecific hybrids (*Vitis vinifera* × *Seyve-Villard*).

The results of this survey supplies suitable data for the dissimilar susceptibilities or tolerances of hybrids of *Vitis vinifera* and of the interspecific hybrids *Vitis vinifera* × *Seyve-Villard* to *M. hapla*. Consequently, these draw attention to the importance of the study of the susceptible as well as the resistant hybrids of grapevine.

Acknowledgements

We thank the Directorate and co-workers of the Research Institute for Viticulture and Ecology for supporting our investigations in the vineyard in the Experimental Station, Miklóstelep.

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 359–366 (1991)

RESISTANCE OF SOME PEACH CULTIVARS AND OF *PRUNUS DAVIDIANA* TO *CYTOSPORA CINCTA*

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(Received: 27 June, 1990; accepted: 2 November, 1990)

The paper presents the results of a single inoculation of *Prunus davidiana* and 16 peach cultivars, and a thrice-repeated inoculation of 8 cultivars with *Cytospora cincta*. The susceptibility of the cultivars is determined by the length of the necrotic spot developed in response to the infection. The data are evaluated by analysis of variance, the significant difference between the cultivars is established by the help of a LSD 5% value. The high environment dependence of resistance to *Cytospora cincta* has been confirmed. According to the results of the experiment *Prunus davidiana* showed in each case the lowest suspectibility, significantly differing from a number of cultivars. Among the peach cultivars 'Sunshine' excelled in resistance.

Keywords: resistance, *Cytospora*, *Prunus*

Introduction

The sudden destruction of apricot and peach trees is rightly called apoplexy, since the previously blossoming trees often promising a good yield die almost overnight. According to the majority of authors, the destruction is caused by *Cytospora cincta* as the primary pathogen (De Fago, 1935; Masten 1958; Tasnády and Lehoczky, 1966; Wensley, 1966). The disease occurs in Europe, in the United States and in Canada alike.

The most efficient means of controlling the pathogen would be the use of cultivars resistant to *Cytospora cincta*. To be able to produce such cultivars we had to determine what response the cultivars that were used in our breeding work would give to the inoculation, and where the source of resistance can be sought.

These are the questions we tried to answer by a series of experiments, the results of which are given in this paper.

Materials and methods

In the course of testing for susceptibility to *Cytospora cincta* in the Winter of 1988–89, the following peach cultivars and hybrids were inoculated: 1000/1 (Nectared-4 × Halehaven), 1000/4 (Nectared-4 × Royal de Cardwell), Suncrest, Sunshine, Szocsnij, 45/68 (seedling of

unknown origin), Nectared-4, *Prunus davidiana*. Besides those listed above, we examined in the Winter of 1987–88 Sz-V-2 (a seedling of unknown origin), 97/68/1 (Ford × Mayflower), 97/68/3 (Ford × Mayflower, II/2 (*P. davidiana* seedling from free pollination), 1000/2 (Nectared-4 × Halehaven), 1000/3 (Nectared-4 seedling from self-pollination), Sunhigh and Nectared-8. These peach cultivars were included in our crossing programme because of various favourable qualities.

The shoots used for the inoculation were collected on 20th December, 1987, 12th December 1988, 20th January and 20th February 1989, and were taken from a nine-year-old variety collection maintained with traditional cultivation technology. From each cultivar 50 shoots, about 30 cm in length and 0.4–0.8 cm in diameter were used. A sample contained shoots from more than one trees of the same cultivar.

The inoculation was performed with *Cytospora cincta* culture isolated from diseased trees and maintained on potato dextrose (PDA) medium (Rozsnyai, 1977).

On the shoots a circular wound of 5 mm diameter was made, and an agar disc of the same size, infected with *Cytospora cincta* was placed on it. The infected wound was covered with wet cotton-wool and aluminium foil, which were removed after 14 days. From the infection to the evaluation the shoots were kept in perlite, in a growth chamber at 15 °C for a month.

In the course of evaluating, we removed the bark from the environment of the site of infection, and measured the length of the necrotic spot formed in the phloem. The data were analysed with variance analysis. In case we found on the basis of the variance analysis that the mean values obtained did not come from the same lot, we compared the cultivars in pairs, and on the basis of the significant difference calculated, placed them in groups of different susceptibility.

Results

The variance analysis of the length of necrotic lesions formed in the phloem during the experiment, in response to the inoculation, indicated a significant difference in susceptibility among the peach cultivars. The F value calculated from the means obtained in the four points of time was higher than the value in Tables 1 and 2 given at the $P = 5\%$ level of probability. So we compared the cultivars in pairs, and on the basis of the LSD 5% value calculated determined which peach cultivars showed significant differences at the given date of measuring. The result is shown in Tables 3 and 4.

Owing to the large number of data and the small differences between the cultivars in the 1987 experiment, it would be unreasonable to compare them in every possible pairing. Therefore, in Table 4 we give only the significant differences between *Prunus davidiana* and the peach cultivars.

Results and discussion

The wide scatter of the basic data (Tables 1 and 2) confirms the opinion published in the literature; namely, that the *Cytospora cincta* may give inconsistent results even with a highly circumspect measuring. The wide scatter and the change in the cultivars' susceptibility in the course of repeated inoculations are due to the complex nature of resistance to *Cytospora cincta*. Many factors are known to show close correlation with the response of peach trees to infection by *Cytospora cincta*. Among them, for example, the year (Dhavan-

Table 1

Susceptibility of various peach cultivars and Prunus davidiana to infection by Cytospora cincta
(Average length of necrosis [mm]; measuring data of 1988–89)

Serial number	Cultivar	1. measuring			2. measuring			3. measuring		
		n	average (x)	scatter	*n*	average (x)	scatter	*n*	average (x)	scatter
1.	1000/4	—	—	—	37.00	16.70	7.21	50.00	55.14	20.30
2.	1000/1	38.00	46.50	23.30	20.00	26.05	11.48	53.00	56.04	24.38
3.	Suncrest	40.00	46.60	35.15	21.00	20.24	5.25	56.00	31.61	7.93
4.	Sunshine	50.00	15.48	11.60	41.00	16.34	7.41	48.00	23.69	8.38
5.	Szocsnij	50.00	19.82	14.23	37.00	25.73	11.27	52.00	28.63	11.36
6.	47/68	49.00	28.57	16.11	52.00	23.12	9.90	53.00	26.87	8.81
7.	Nectared-4	49.00	55.20	25.08	—	—	—	50.00	29.44	9.41
8.	*P. davidiana*	49.00	12.71	0.94	38.00	12.84	7.82	28.00	14.50	5.14
		F value calculated: 34.3 *F* value in table: 2.1			*F* value calculated: 11.2 *F* value in table: 2.1			*F* value calculated: 51.6 *F* value in table: 2.0		

tari and Dirks, 1983), the vitality of the tree (Wensley, 1966), the frost tolerance of the cultivar (Rohrbach and Luepschen, 1968) and its callus formation ability (Plamiter and Hickey, 1970) may have great influences on

Table 2

Susceptibility of various peach cultivars and of Prunus davidiana to infection by Cytospora cincta
(Average length of necrosis [mm]; measuring data of 1987)

Serial number	Cultivar	*n*	Average (x)	Scatter
1.	45/68	24.00	26.50	11.46
2.	Sz-V-2	24.00	29.17	8.62
3.	Sunshine	24.00	46.42	20.08
4.	97/68/3	23.00	36.74	14.22
5.	97/68/1	31.00	32.74	11.60
6.	II/2	21.00	35.05	12.81
7.	*P.davidiana*	18.00	23.61	4.91
8.	1000/4	24.00	52.54	22.32
9.	Szocsnij	22.00	28.45	14.35
10.	1000/3	20.00	35.20	18.39
11.	1000/2	22.00	41.77	16.07
12	Nectared-4	24.00	41.75	15.75
13.	Sunhigh	22.00	40.05	19.29
14.	1000/1	24.00	53.58	15.86
15.	Suncrest	24.00	25.58	7.76
16.	Nectared-8	25.00	49.72	22.68

Calculated F value: 8.9
Table value: 1.7

the results of inoculation. Our samples came from more than one trees within each cultivar, and the thickness of the shoots may not have been completely equal either, so the scatter of the data is understandable.

Despite what is described above, the repeated measuring and the statistical analysis may provide a basis for the reliable classification of the peach cultivars. The peach cultivars tested by us are reasonably placed in the following two groups: cultivars of the lowest susceptibility equal to that of *Prunus davidiana*, and those significantly more susceptible than *Prunus davidiana*.

Figures 1–4 show the average length of the necrotic spots having appeared in the peach cultivars examined. The horizontal line indicates the length above which the cultivars are significantly more susceptible than the *Prunus davidiana*. The peach cultivars showing values below the line have low susceptibility equal to that of *Prunus davidiana*.

Table 3

Comparison of various peach cultivars and Prunus davidiana for susceptibility to Cytospora cincta, significance analysis
(on the basis of measuring data of 1988–1989)

Serial number	Cultivar	1. measuring		2. measuring		3. measuring	
		x-x	LSD 5%	x-x	LSD 5%	x-x	LSD 5%
1.	1000/4-1000/1	—	—	+9.35	6.11	0.90	7.35
2.	1000/4-Suncrest	—	—	3.54	5.92	−23.53	7.16
3.	1000/4-Sunshine	—	—	0.36	4.08	+31.45	7.72
4.	1000/4-Szocsnij	—	—	+9.03	4.29	+26.51	7.42
5.	1000/4-45/68	—	—	+6.41	3.67	+28.27	7.35
6.	1000/4-Nectared-4	—	—	—	—	+25.70	7.57
7.	1000/4-*P. davidiana*	—	—	3.86	4.23	+40.64	10.54
8.	1000/1-Suncrest	0.10	20.57	5.81	7.75	+24.43	6.95
9.	1000/1-Sunshine	+31.00	18.56	+9.71	5.90	+32.35	7.51
10.	1000/1-Szocsnij	+26.68	18.56	0.32	6.11	+27.40	7.21
11.	1000/1-45-68	17.93	18.73	2.93	5.49	+29.17	7.14
12.	1000/1-Nectared-4	8.70	18.73	—	—	+26.60	7.35
13.	1000/1-*P. davidiana*	+33.79	18.73	+13.21	6.06	+41.54	10.32
14.	Suncrest-Sunshine	+31.12	18.04	3.90	5.71	+7.92	7.32
15.	Suncrest-Szocsnij	+26.78	18.04	5.49	5.92	2.97	7.01
16.	Suncrest-45/68	18.03	18.20	2.88	5.30	4.74	6.95
17.	Suncrest-Nectared-4	8.60	18.20	—	—	2.17	7.16
18.	Suncrest-*P. davidiana*	+33.89	18.20	+7.40	5.87	+17.11	10.13
19.	Sunshine-Szocsnij	4.34	16.03	+9.39	4.08	4.95	7.58
20.	Sunshine-45/68	13.09	16.20	+6.77	3.46	3.18	7.51
21.	Sunshine-Nectared-4	+39.72	16.20	—	—	5.75	7.72
22.	Sunshine-*P. davidiana*	2.77	16.20	3.50	4.02	9.19	10.70
23.	Szocsnij-45/68	8.75	16.20	2.61	3.67	1.77	7.21
24.	Szocsnij-Nectared-4	+35.38	16.20	—	—	0.81	7.42
25.	Szocsnij-*P. davidiana*	7.11	16.20	+12.89	4.23	+14.13	10.39
26.	45/68-Nectared-4	+26.63	16.36	—	—	2.57	7.35
27.	45/68-*P. davidiana*	15.86	16.36	+10.27	3.61	12.37	10.32
28.	Nectared-4-*P. davidiana*	+42.49	16.36	—	—	+14.94	10.54

The difference is significant at $\pm$ P = 5%.

The results of the experiments conducted in 1987 indicated that 13 of the 16 cultivars examined showed no difference in susceptibility to the infection (Fig. 4). Apart from *Prunus davidiana* only the cultivar 'Sunshine' could on each occasion be placed in the low susceptibility category. The hybrids 1000/1 and 1000/4 can be ranked with the most susceptible cultivars.

The above results support the opinion that the peach cultivars have but a low resistance to *Cytospora cincta*, and their susceptibility largely depends on environmental factors (Luepschen, 1981). Examinations of various *Prunus* species have pointed out that they are more or less suspectible, only *P. gracea*

Table 4

Comparison of Prunus davidiana and various peach cultivars for susceptibility to Cytospora cincta, significance analysis
(on the basis of measuring data of 1987)

Serial number	Cultivars	x-x	LSD 5%
1.	*Prunus davidiana* — 45/68	2.90	23.72
2.	*Prunus davidiana* — Sz-V-2	5.56	23.72
3.	*Prunus davidiana* — Sunshine	22.81	23.72
4.	*Prunus davidiana* — 97/68/3	13.13	24.16
5.	*Prunus davidiana* — 97/68/1	9.13	21.42
6.	*Prunus davidiana* — II/2	11.44	25.17
7.	*Prunus davidiana* — 1000/4	+28.90	23.72
8.	*Prunus davidiana* — Szocsnij	4.84	24.64
9.	*Prunus davidiana* — 1000/3	11.59	25.75
10.	*Prunus davidiana* — 1000/2	18.16	24.64
11.	*Prunus davidiana* — Nectared-4	18.14	23.72
12.	*Prunus davidiana* — Sunhigh	16.43	24.64
13.	*Prunus davidiana* — 1000/1	+30.00	23.72
14.	*Prunus davidiana* — Suncrest	1.97	23.72
15.	*Prunus davidiana* — Nectared-8	+26.10	23.31

The difference is significant at $\pm$ P = 5%.

and *P. sieboldii* proved resistant (D. Rozsnyai et al., 1972; Kern, 1955). On the basis of our data *Prunus davidiana* cannot be regarded as totally resistant, though in comparison with the peach cultivars it is undoubtedly practically resistant.

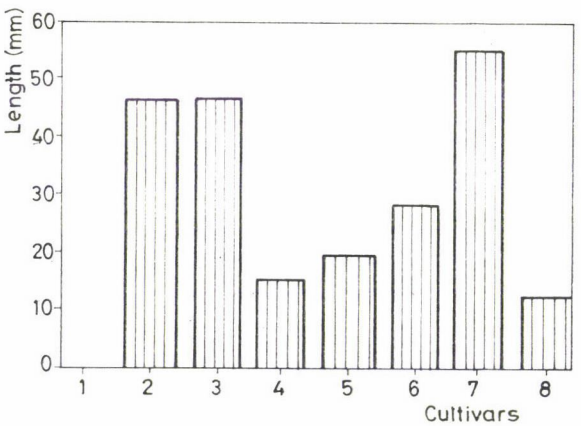

Fig. 1. Susceptibility of various peach cultivars and of *Prunus davidiana* to infection by *Cytospora cincta* (Average length of necrosis (mm) 1988–1989. 1st measuring)
(Order of cultivars as in Table 1)

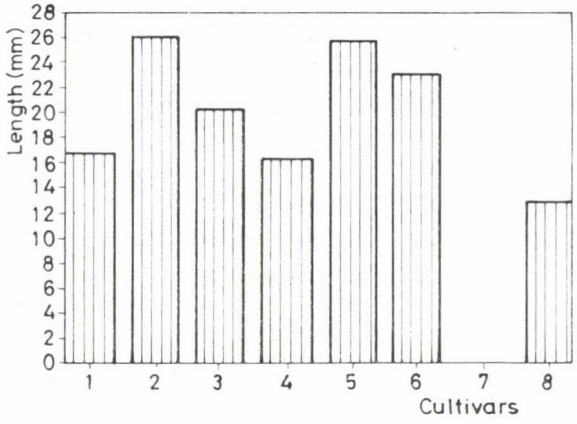

Fig. 2. Susceptibility of various peach cultivars and of *Prunus davidiana* to infection by *Cytospora cincta* (Average length of necrosis (mm) 1988–1989. 2nd measuring)

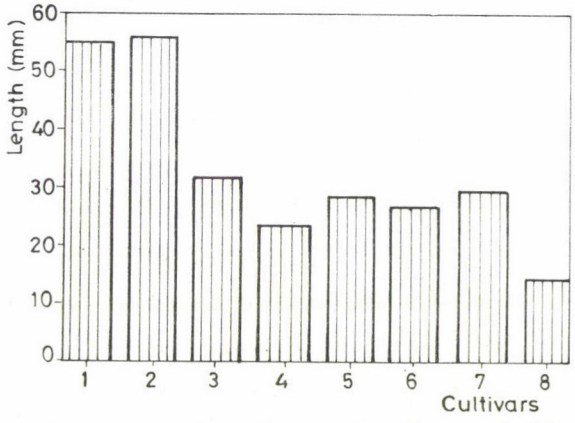

Fig. 3. Susceptibility of various peach cultivars and of *Prunus davidiana* to infection by *Cytospora cincta* (Average length of necrosis (mm) 1988–1989. 3rd measuring)
(Order of cultvars as in Table 1)

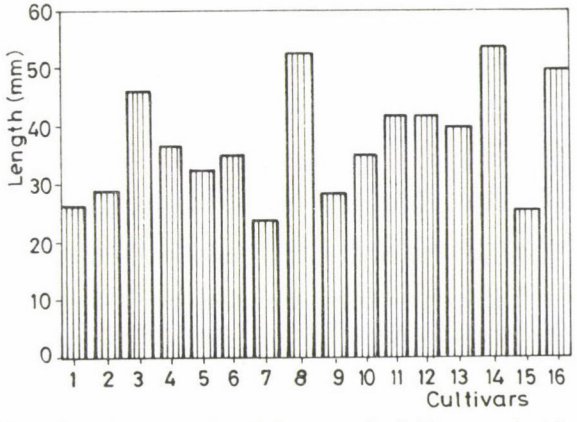

Fig. 4. Susceptibility of various peach cultivars and of *Prunus davidiana* to infection by *Cytospora cincta* (Average length of necrosis (mm). Measuring in 1987)
(Order of cultivars as in Table 2)

The increased resistance of cultivar 'Sunshine' might be explained by its origin from the bud mutation of the cultivar 'Redhaven' and according to same authors 'Redhaven' can be placed among the resistant cultivars (Luepschen et al. 1975; Luepschen 1981).

Because of the earlier mentioned complexity of the *Cytospora cincta* resistance, the extent of susceptibility established by inoculation does not necessarily correspond to the facts experienced in a given plantation, where the trees are exposed to various environmental effects which influence the resistance. Nevertheless, the method is by all means suitable for picking out the extreme types.

Out of the peach varieties examined by us increased attention must be paid to *P. davidiana* and the cultivar 'Sunshine' in peach breeding programmes which include breeding for resistance to *Cytospora cincta*.

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 367–375 (1991)

PLANT GROWTH, METABOLISM AND ADAPTATION IN RELATION TO STRESS CONDITIONS. XI. MODIFICATION OF OSMOTIC STRESS-INDUCED METABOLIC EFFECTS BY GA_3 OR IAA IN *PISUM SATIVUM* L. PLANTS

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(Received: 28 March, 1990; accepted: 27 June, 1990)

The antagonistic effects of either GA_3 or IAA on salinity induced stress on various metabolic activities in *Pisum sativum* plants at different stages of development were investigated.

Seed presoaking in GA_3 or IAA appeared to nullify either partially or completely the effects induced by the different levels of salinity on protein-N, total soluble-N and proline contents in shoots and roots at all stages of plant development.

GA_3 and IAA appeared also to nullify the accumulation of Na ions, ABA and at the same time increased the citric and oxalic acid contents.

It is suggested that GA_3 or IAA may provide some salt tolerance at the metabolic level by its capacity to restore the synthesis of various metabolites and to inhibit the synthesis of others.

Keywords: *Pisum sativum*, salinity, GA_3, IAA, nitrogen, proline, acids, Na^+, ABA

Introduction

The response of crop plants to salinity stress is generally reflected in decreased growth and productivity. This inhibited growth has previously been ascribed to the metabolic disorders associated with the profound hormonal changes in the plants subjected to salinity stress (see Itai and Vaadia, 1971; Hsiao, 1973; Aharoni and Richmond, 1978; Davenport et al., 1980; Wang et al., 1984; Younis et al., 1987 a and b; Abo-Hamed et al., 1990; El-Shahaby et al., 1990).

The most important aspect of this research is the possibility that hormonal regulation is involved in the control of water potential and membrane permeability in plants and thus in control of water deficit. Consequently, certain trials have been made to improve salt tolerance using CCC, GA_3 and IAA (Gabr et al., 1977; Salama et al., 1981; Heikal et al., 1982; Banyal and Rai, 1983; Vidhu and Murty, 1985; Younis et al., 1989).

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Abbreviations: GA_3, gibberellic acid; IAA, indoleacetic acid; ABA, abscisic acid; CCC, (2-chloroethyl)-trimethyl ammonium chloride.

Although promising results were obtained, much more experimentation is needed in attempting to adapt plants to grow under stress conditions. In this investigation one species of economical importance, *Pisum sativum*, was chosen in an attempt to improve its salt tolerance by treatment with GA_3 or IAA. The approach applied was to investigate the changes in nitrogen metabolites, due to the role played in part by proline and other soluble nitrogen fractions in plants under stress conditions. Furthermore, changes in the contents of organic acids, Na^+ and ABA were determined to substantiate the view that retention of the hormonal balance in plant tissue is considered to be an assurance of normal plant growth.

Materials and methods

Pisum sativum L. (cv. Little Marvel) seeds were surface sterilized and divided into 3 groups that were then soaked in distilled water, GA_3 (100 mg/l) or IAA (25 mg/l); soaking being carried out for one hour at room temperature (20 °C) and in the dark. As in Younis et al. (1989), these concentrations of hormoned were the most effective in eliminating the harmful effects of salinity on the developing plants. Afterwards, the seeds were germinated in water for 7 days and then transferred to culture solutions as described by El-Shahaby et al. (1990).

Each group of seedling was divided into 3 sub-groups and treated with different levels of salinity at (A) the vegetative stage (14-day-old), (B) the flowering stage (26-day-old) and (C) the fruiting stage (36-day-old). At each stage, *Pisum sativum* plants were transferred to the experimental nutrient solution with different levels of NaCl (−0.3, −0.6, −0.9 and −1.2 MPa).

The plants were allowed to grow indoors in the laboratory and sampling was carried out 4 days after each treatment.

Analytical methods

The total soluble- and protein-N were determined by the conventional micro-kjeldahl method as described by El-Shahaby et al. (1990): The content of proline was determined by the method adopted by Bates et al. (1973).

The methods adopted for determination of citric and oxalic acids were essentially those described by Hasaneen et al. (1987).

Abscisic acid was extracted from the shoots and separated as in Shindy and Smith (1975), and bioassayed as recommended by Wright (1969) using the straight growth test of *Triticum* coleoptile segments.

Na^+ was determined in the oven dried samples that were digested in HNO_3 and made up to volume. Flame emission spectrophotometry was used to measure Na^+ concentration as in Abo-Hamed et al. (1990).

Triplicate samples were always taken for analyses and the data were statistically analysed using the least significant difference (LSD.) at the 1% and 5% probability levels.

Results

1. *Changes in nitrogen content: (Fig. 1)*

(a) *Total soluble-N.* At the vegetative, flowering and fruiting stages, the presoaking of seeds in GA_3 or IAA induced increases in the total soluble-N content of shoots and roots of the plants treated with salinity solutions of

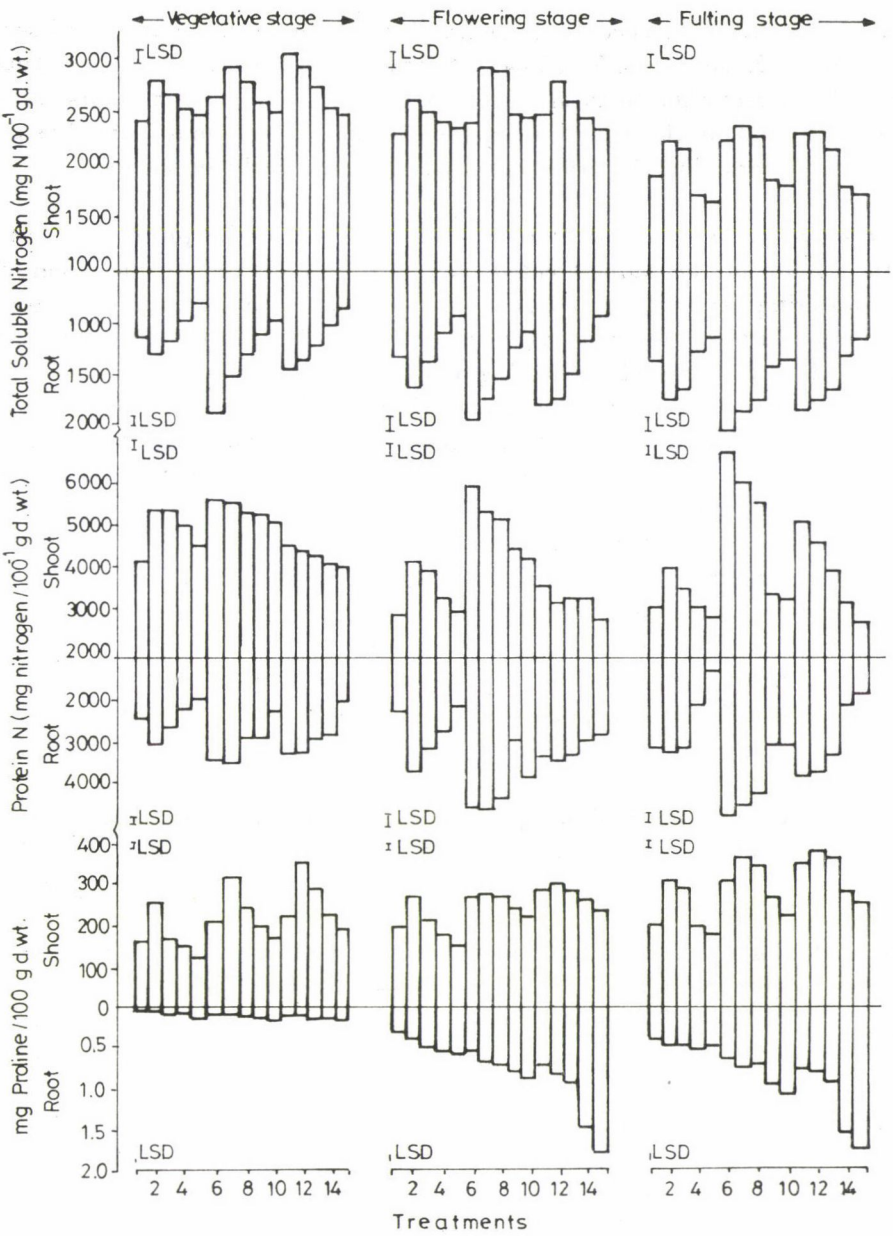

Fig. 1. Effect of seed presoaking in GA_3 or IAA on nitrogen content of *Pisum sativum* plants, stressed with salinity at different stages of plant development. In this and the following figures treatments were as follows:

1-Non-salinized	6-GA_3-pretreated	11-IAA-pretreated
2-NaCl-0.3 MPa	7-GA_3 + NaCl (−0.3MPa)	12-IAA + NaCl (−0.3 MPa)
3-NaCl-0.6 MPa	8-GA_3 + NaCl (−0.6 MPa)	13-IAA + NaCl (−0.6 MPa)
4-NaCl-0.9 MPa	9-GA_3 + NaCl (−0.9 MPa)	14-IAA + NaCl (−0.9 MPa)
5-NaCl-1.2 MPa	10-GA_3 + NaCl (−1.2 MPa)	15-IAA + NaCl (−1.2 MPa)

—0.3 and —0.6 MPa, as compared with plants from hormone-untreated seeds. On the other hand, at —0.9 and —1.2 MPa salinity, GA_3 or IAA pretreatment nullified the reduction in total soluble-N that was observed in plants treated with salinity alone. It was also apparent that GA_3 was more effective than IAA in counteracting the effects of salinity particularly in shoots.

(b) *Protein-N.* As apparent from Fig. 1, increases in the protein-N content in the shoots of the saline-treated plants, were observed at the vegetative and flowering stages in response to presoaking seeds in GA_3. In contrast, a reduction in protein-N content in shoots was elicited, in the different saline-treated plants, after seed presoaking in IAA.

At the fruiting stage, the shoots of the variously saline-treated plants from GA_3 or IAA pretreated seeds showed highly significant increments in protein-N contents above those values in plants from hormone-untreated seeds, except in the plants treated with salinity at —0.9 and —1.2 MPa which respectively showed either a non-significant increase or a highly significant decrease in protein-N as a result of presoaking seeds in IAA.

In roots, the protein-N content showed increments in the plants treated, at the vegetative stage, with salinity at —0.3 MPa under the effect of seed presoaking in GA_3 or IAA. Moreover, as compared with non-salinized values, the observed decreases in protein-N content in the saline-treated plants were either reduced or totally nullified in response to presoaking the seeds in GA_3 or IAA.

At the flowering and fruiting stages, pretreatment of seeds with GA_3 or IAA induced increases in the protein-N contents in the roots of the plants treated with NaCl at —0.3 and —0.6 MPa above those values in hormone-untreated plants. This pretreatment, however, induced either total elimination (with GA_3) or a reduction (with IAA) of the observed reduction in the protein-N contents induced by —0.9 and —1.2 MPa salinity.

In this context it should be mentioned that, at all stages of plant growth and development, the pattern of changes in total -N in shoots and roots of the hormone-treated plants was comparable to that of protein-N which constituted a predominant fraction in the nitrogen pool of pea plants.

(c) *Changes in proline content.* The presoaking of seeds in GA_3 or IAA induced increases in the proline content of pea shoots and roots, compared with the amounts of proline in the saline-treated plants, in which highly significant increases were observed at all stages of plant development. The magnitude of response was more pronounced with IAA than with GA_3. Furthermore, the above mentioned response was more obvious with the lower than with the higher levels of salinity.

2. *Changes in organic acids content: (Fig. 2)*

(a) *Oxalic acid.* The presoaking of seeds in GA_3 or IAA induced increases in oxalic acid content in both shoots and roots of plants treated with —0.3 MPa saline solutions at the vegetative stage. With —0.6 MPa salinity treatment, the induced reduction in oxalic acid content was completely absent in response to seed presoaking in GA_3 or IAA. Also the observed reductions in oxalic acid content, in response to salinity treatment at —0.9 and —1.2 MPa, were reduced by seed pretreatment with GA_3 or IAA.

The presoaking of seeds in GA_3 or IAA induced increases in oxalic acid content in both shoots and roots of plants treated at the flowering stage with salinity of —0.3 and —0.6 MPa. In comparison with hormone-untreated plants, the presoaking of seeds in the solutions of growth regulators reduced the observed reduction in the acid content in shoots and roots of the plants treated with salinity of —0.9 and 1.2 MPa.

At the fruiting stage, further increases were elicited in oxalic acid in both shoots and roots of the saline-treated plants (at —0.3 MPa) after the presoaking the seeds in GA_3 or IAA. In contrast, this pretreatment appeared to induce slight changes in oxalic acid content from those values detected in shoots and roots of plants treated with salinity of —0.6, —0.9 and —1.2 MPa.

(b) *Citric acid.* The effect of seed presoaking in GA_3 or IAA on citric acid content in pea shoots and roots of the plants treated with different salinity solutions at any stage of development was, in general, similar to that obtained for oxalic acid content.

3. *Changes in sodium content: (Fig. 3)*

In relation to the non-salinized control values, the accumulation of Na^+ in both shoots and roots in response to the different salinity treatments, was reduced when the seeds were presoaked in GA_3 or IAA; the magnitude of reduction was more pronounced with GA_3 than with IAA at all stages of plant development.

4. *Changes in abscisic acid content: (Fig. 4)*

During each stage of plant development, a greater reduction in ABA content of shoots of —0.3 MPa saline-treated plants was elicited in response to presoaking seeds in GA_3 or IAA. On the other hand, the observed increases in ABA content in —0.6, —0.9 and —1.2 MPa saline-treated plants were either reduced or completely nullified by the pretreatment of seeds with GA_3 or IAA.

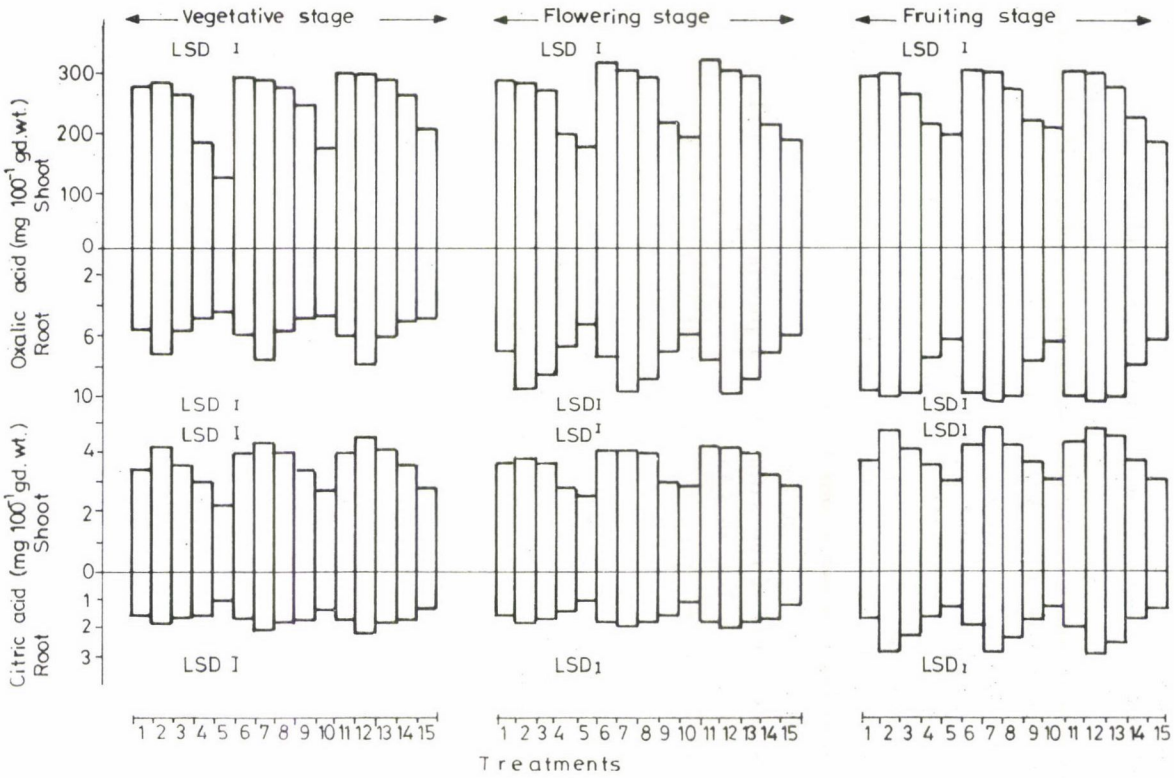

Fig. 2. Effect of seed presoaking in GA_3 or IAA on organic acids content of *Pisum sativum* plants, stressed with salinity at different stages of plant development. Treatments 1 to 15 as in *Fig. 1.*

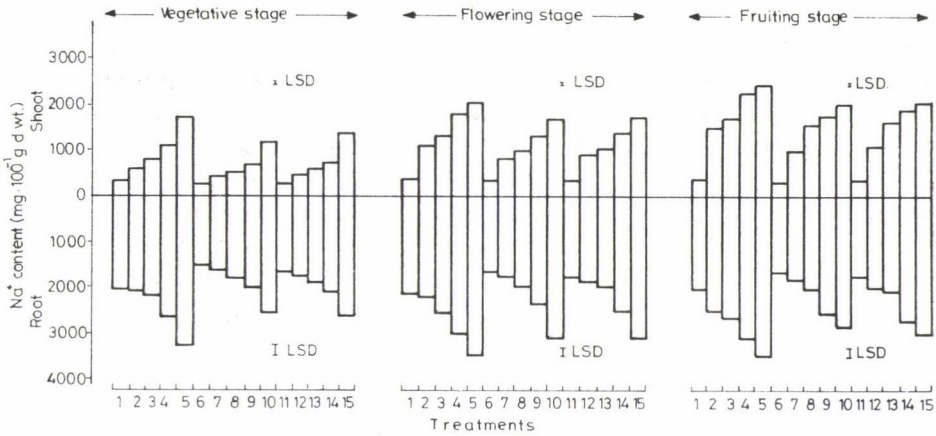

Fig. 3. Effect of seed presoaking in GA_3 or IAA on sodium content of *Pisum sativum* plants, stressed with salinity at different stages of plant development. Treatment 1 to 15 as in *Fig. 1.*

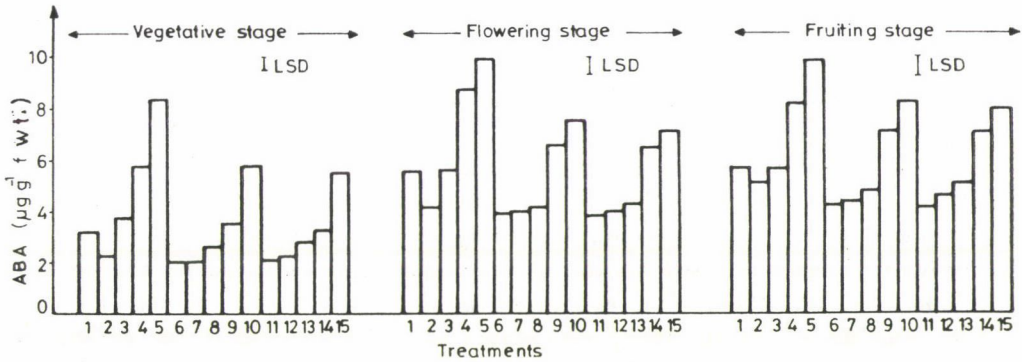

Fig. 4. Effect of seed presoaking in GA_3 or IAA on abscisic acid content of *Pisum sativum* shoots, stressed with salinity at different stages of plant development. Treatments 1 to 15 as in *Fig. 1.*

Results and discussion

El-Shahaby et al. (1990) have shown that the total amount and the relative composition of the nitrogen pool were altered by salinity and it seems that these changes were dependent mainly on (1) the level of salinity used, (2) the stage at which plants were treated and (3) the plant organ.

The results reported here show that the presoaking of *Pisum sativum* seeds in either GA_3 or IAA appeared to nullify, either partially or completely, or to augment the observed effects of salinity on the protein-N, total soluble-N and proline contents at all stages of plant development. These results appear to coincide with the recovery in the growth of pea plants under these hormonal treatments (Younis et al., 1989). Similarly Banyal and Rai (1983) indicated

that the GA_3-mediated recovery of hypocotyl growth in *Brassica campestris* under osmotic stress, may be a consequence of increased protein and RNA content. Thus, the observed increase in proline and other nitrogen metabolite contents due to GA_3 or IAA seed pretreatment can be used as an indicator in the selection of seed for plants that will withstand saline stress through osmoregulation, as reported by Singh et al. (1972), Imamul Huq and Larher (1983), El-Shahaby et al. (1990) and Abo-Hamed et al. (1990).

The beneficial effect of seed presoaking in GA_3 or IAA on citric and oxalic acid contents, in shoots and roots of the saline-treated plants, was manifested by augmentation of the increases and nullification of the reductions observed in the plant contents of these acids. This was associated with reducing the injurious accumulation of Na ions in shoots and roots. Thus seed presoaking in GA_3 or IAA appeared to nullify the accumulation of Na ions and at the same time to increase the organic counter ion content. These results agree with the data obtained by Darra and Saxena (1973) and Heikal et al. (1982). The connection between the effects of seed presoaking in GA_3 or IAA on organic acids and sodium contents led us to conclude that GA_3 or IAA may provide the saline treated plants with some tolerance of salinity stress. The magnitude of the response was dependent upon the level of the salinity used.

The results of this investigation also indicate that seed presoaking in GA_3 or IAA stimulated the reduction in the levels of ABA in shoots of the salinized plants, and this could possibly be a reason for the promotion in growth of these stressed plants, as previously demonstrated by the authors (Younis et al., 1989). This conclusion is supported by the results obtained by Sinel'nikova et al. (1972) who stated that the removal of the growth inhibiting effect of NaCl salinization after GA_3 treatment is associated with changes in the ratio of stimulators to inhibitors in the plant tissue.

It became evident from this study that the exogenous application of GA_3 or IAA appeared to supply more or less sufficient quantities which were implicated in the recovery of growth under conditions of osmotic stress. This recovery may be a consequence of several roles played by such hormones which can cause triggering of the internal cellular metabolism and also induce alterations in the ratios of the growth regulators which have been shown to be critical determinators of growth and differentiation (Street and Cockburn, 1970).

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 377–381 (1991)

REMOVAL OF LINDANE RESIDUES FROM APPLE BY COMMERCIAL PROCESSING AND PRESERVATION OPERATIONS

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(Received: 28 March, 1990; accepted: 15 June, 1990)

Commercial processing and preservation operations of plant foods remove major portions of the toxic residue of chemicals that are currently permitted on the raw agricultural crops. In this work, gas-liquid chromatography with electron capture detector (ECD) was used to determine the extent reduction of Lindane residue levels found on and in contaminated plant foods by the effect of home preparation procedures and technological processes directly after Lindane application and fifteen days later. Simple washing with tap water and washing with detergent reduced Lindane residues to 74.7% and 35.1% of its initial value immediately after Lindane application, and to 75.5% and 60.8% after 15 days post-application. Washing plus peeling reduced 30% and 49.7% of the initial Lindane residue immediately after application and 15 days later, while washing plus peeling plus processing to jam reduced 85.8% and 91% of the Lindane residues found on unwashed fruits at the same mentioned intervals. A waiting period of 15 days after Lindane application brought a significant reduction of residues found on unwashed apples, as only 46.1% of that residue was found after immediate application. Sterilization and pasteurization of fresh apple juice reduced 46.1% and 56.3% of Lindane residues directly after application, and 51.9% and 78.7% after 15 days of Lindane application. Preservation of peeled fruits as an apple jam decreased the Lindane residues by 79.7% and 61.1% immediately after Lindane application and 15 days later.

Keywords: apple, gas-liquid chromatography, Lindane, pesticide residue, washing

Introduction

Currently, the use of pesticides in the agriculture fields has provided numerous benefits in terms of increased production and quality of the product. At the same time, pesticides are toxic chemicals or in fact are poisons, and to avoid the toxic effects or to protect the health of human beings, most countries have introduced laws governing not only the use of pesticides, but also setting limits for the levels of pesticide residues which may be tolerated in foods (Maybury, 1989). The National Food Processors Association (NFPA) has long been interested in the effect of commercial processing on pesticide residues in foods, and it represents nearly 600 companies including most of the major food processors in the United States. The mission of NFPA is to serve the food processing industry and consumers by helping to assure the safety, wholesomeness, and nutritional value of the nation's food supply and also to show that processing almost always considerably reduces residue levels

(Elkins 1989). It was necessary to confirm that the fresh fruits and vegetables available for public consumption must be free of excessive pesticide residues. Tolerances are the best way to avoid uncertainty about the safety of pesticide residues in food, and, therefore, to assist both the food processing industry and consumers, desired residue levels can be achieved in the food supply by using various food processing methods (Trichilo and Schmitt 1989).

Materials and methods

Reagents

Lindane (α-Hexachlorocyclohexane) technical grade material is specified to be 99.9% pure with light white colour, Acetone pure, Benzene pure, Dichloromethane (Merck No. 6050), n-hexane (Merck No. 9688), Elution mixture (10:2:2) dichloromethane: benzene: acetone, Charcoal pure (AR) (No. 2186), Cellite 545, C. Roth Karlsruhe, Silica gel 0.05–0.2 mm (Merck No. 7734), Sodium Chloride (AR) and anhydrous Sodium Sulphate.

Equipment

A Hewlett–Packard Model 5730/A gas-liquid chromatograph, equipped with a linear Ni^{63} electron capture detector (18713/A) and an all-glass transfer line from the column to the detector, was used for the Lindane assays. A 1.5 mm (i.d.) $\times$ 6 feet glass column packed with 1.95% OV-17 on 100–120 mesh Chromosorb was used under the following conditions: Carrier gas 5% methane in argon at 60 cm^2/min flow, injection port 250 °C, Oven temp. 270 °C, Detector temp. 300 °C, Starting temp. 200 °C, Speed of heating 2 °C/min. and initial holding time 2 min. Injection of 1 μl using 10-μl Hamilton syringe equipped with change adapter.

Treatment

Ten kilograms of mature apples (Budapest markets) were sprayed with Sigma Ultra Sprayer using the field concentration of 0.6 g/liter Lindane. The treated fruits were left to dry in plastic trays and loosely covered with polyethylene bags. Treated fruits were divided into two equal parts, the first part used directly after treatment and the second one kept for 15 days at 10 °C to simulate shopping and marketing conditions. Fruits of both parts were washed with water only and with detergent, and washed fruits processed using sterilization and pasteurization, were preserved as an apple jam. The same steps as in home preparation and other preservation methods were done for untreated apples.

Extraction and clean-up

Lindane residues are extracted from treated apples (100 g sample) with 200 ml acetone and the extract filtered. Then, 50 ml of the filtration product was diluted with 200 ml of water and dichloromethane (1:1) for partitioning the residues. The extract was dried with anhydrous sodium sulphate and dichloromethane layer was kept. The dichloromethane layer was purified through a charcoal-silica gel clean-up column with the eluation mixture (dichloromethane : benzene : acetone, (10:2:2). Then, the solvent mixture was evaporated and the Lindane residues dissolved again in 10 ml of n-hexane and diluted to 6-times in n-hexane for chromatographic determination (Tag El-Din 1987).

Recovery value of Lindane

The efficiency of the gas liquid chromatographic method for residue determination of Lindane was achieved by adding 100 μg Lindane/g sample to a portion of the untreated apple sample which was then put through the extraction, clean-up and residual determination as followed in the used methods. The average recovery value for Lindane in apple was 86.4%.

Results and discussion

Removal of Lindane residues by washing and peeling

The removal of pesticide residues from plant foods by the effect of commercial washing is controlled by many factors, such as chemical structure, chemical properties of the pesticide, nature of the food commodity and duration of time that the compound has been in contact with the food, also by the formulation in which the pesticide was applied and the weather conditions as stated by Elkins (1989). Table 1 shows the effect of simple washing, complete washing with detergent, peeling and waiting period for 15 days after Lindane application. A simple washing caused 25.3% reduction in Lindane residues from 123.8 μg/kg to 92.5 μg/kg immediately after applica-

Table 1

Effect of simple washing, adequate washing, peeling, and waiting period on Lindane residue levels on apples

Home preparation techniques	Lindane residues "ug/kg"				
	Immediately after treatment		15 days after treatment		Effect of waiting period
	residues mean ± LSD	reduction %	residues mean ± LSD	reduction %	15 days residues / initial residues ×100
Unwashed fruits	123.8±6.8	00.00	57.1±8.1	00.00	46.06
Simple* washing	92.5±3.1	25.28	43.1±8.6	24.47	46.56
Adequate** washing	43.4±3.6	64.94	43.7±7.7	39.23	79.84
Peeling washing	86.7±7.7	29.97	28.7±1.8	49.65	33.11

* Simple washing: Washing with tap water only.
** Adequate washing: Washing with detergent.

tion, and washing with detergent resulted in 64.9% reduction in Lindane residues immediately after application from 123.8 μg/kg. A simple washing after 15 days of Lindane application caused 24.5% reduction in residue level from 57 μg/kg to 43.1 μg/kg, while washing with detergent caused 39.2% reduction from 57 μg/kg to 34.7 μg/kg for the same mentioned interval. Peeling plus washing reduced 30% and 49.7% of the initial Lindane residue level immediately after application and fifteen days later, from 123.8 μg/kg to 86.7 μg/kg and from 57 μg/kg to 28.7 μg/kg, respectively, while peeling alone reduced 4.7% and 25.2% in Lindane residue level at the same mentioned intervals. Washing plus peeling plus processing apples into jam caused a significant reduction for the initial Lindane residues found on unwashed

apples, reached to 85.8% and 91% directly after Lindane application and fifteen days later. These findings agree with those of Lamb et al. (1968), which established that commercial washing of potato treated with DDT removed about 20% of total DDT residues, while home preparative procedures and peeling of treated potato removed more than 91% of DDT residue level. Also, washing plus blanching plus canning of treated tomato fruits removed 99% of malathion and carbaryl residues, as stated by Elkins (1989). A waiting period of 15 days after Lindane application is most effective in reducing the residue level from 123.8 μg/kg to 57 μg/kg. These obtained results agree completely with those of Farrow et al. (1968 and 1969), Hemphill et al. (1967), Fahey et al. (1969), Talekar et al. (1977) and Bessar (1984).

Removal of Lindane residues by some preservation processes

The influence of various technological processes for fresh apple juice on Lindane residues is shown in Table 2. The obtained results emphasized that pasteurization and sterilization of fresh apple juice reduced residue levels from 92.5 μg/kg to 49.9 μg/kg and 40.4 μg/kg; with reduction percent of 46.1% and 56.3% of the initial Lindane residue in fresh apple juice immediately after application. A similar effect was noticed after 15 days of Lindane application, where residue levels decreased from 43.1 μg/kg to 20.7 μg/kg and 9.2 μg/kg with reduction percent of 51.9% and 78.7% by pasteurization and sterilization, respectively. Also, the preservation of peeled apples by an apple

Table 2

Effect of various technological processes on Lindane residues in apple products

Preservation techniques	Lindane residues "ug/kg"				
	Immediately after treatment		I5 days after treatment		Effect of waiting period
	residues mean ± LSD	reduction %	residues mean ± LSD	reduction %	$\frac{\text{15 days residues}}{\text{initial residues}} \times 100$
Fresh juice	92.5±0.052	00.00	43.07±0.023	00.00	46.56
Pasteurized juice	49.9±0.018	46.05	20.70±0.005	51.94	41.48
Sterilized juice	40.4±0.004	56.32	09.18±0.003	78.69	22.72
Peeled juice	86.7±0.017	00.00	28.71±0.004	00.00	33.11
Apple jam	17.6±0.004	79.70	11.16±0.002	61.13	63.41

The data are mean values and standard deviations of five parallels.

jam has a significant effect on the reduction of Lindane residue levels. Lindane residues lowered from 86.7 μg/kg to 17.6 μg/kg with reduction percent of 79.7% immediately after Lindane application, and from 28.7 μg/kg to 11.2 μg/kg with reduction percent of 61.1% 15 days after the Lindane application. These obtained data strongly support the view that, despite the high resistance of Lindane to degradation by many physical factors, heat treatments, pasteurization and sterilization removed the major portions of Lindane residues adding to the effective role of washing and peeling as previously mentioned. The waiting period of fifteen days has an important role in the removal and reduction of pesticide residue levels.

Our obtained data are in completely harmony with these findings of many authors, e.g., Koivistoinen et al. (1964), Carlin et al. (1966), Farrow et al. (1968 and 1969), Elkins et al. (1968), Newsome (1980), Talekar et al. (1977), Bessar (1984) and Elkins (1989).

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 383–396 (1991)

Plant cultivation

EFFECT OF N-DISTRIBUTION ON INTENSIVELY PLANTED HAYFIELDS

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(Received: 13 December, 1988; accepted: 30 March, 1989)

The various ways of distributing 300 kg/ha N influence the annual yield of hay and the seasonal output of cutting. The autumn distribution of N is not advisable. N fertilization is most important in spring, because its omission causes yield loss. By the proper application of the N-fertilizer the yearly distribution of the yield can be influenced. The balanced yield level of the successive growths was ensured by the even distribution of the N-fertilizer. The annual amount and distribution of precipitation determined the volume of yield and influenced the effectiveness of the N-fertilizer. The proper distribution of N increased the N content of the grassland. N-doses supplied for different growths showed various effectiveness.

Keywords: grassland, hayfield, fertilization, N-distribution

Introduction and Literary review

A large amount of Hungarian and international data are available concerning the distribution of N over the sections of grazing lands. However, little is known of the distribution of N on hayfields. Seasonal changes in the yields of grasslands must also be reckoned with in Hungary. The production potential of the grassland, the number and yield of the successive growths, determine the number and time of cuttings on the basis of those crops that are economically worth cutting.

From intensively planted hayfields the yield of grass is larger. Therefore, the way of distributing the larger N quantity applied may influence the volume of yield and its distribution over the vegetation period.

Some have tried to balance the seasonal yield fluctuation of the grassland by different ways of distributing the N-fertilizer, and have studied the possibility of obtaining larger yields. Klapp (1952) used 3 variations of distributing 240 kg/ha N (given once before the vegetation period; distributed at 4 equal rates over the whole year; 1/6 of N supplied at the beginning of the vegetation period, 1/3 of it in summer and half of it at the end of the season). The latter forms of N-distribution resulted in a more balanced yield during the year. Schulze (1954, 1956) experimented similarly with various forms of N distribution: giving the whole dose of N at the beginning of the season; dis-

tributing it everly; supplying no N in early spring but distributing 1/6 of it late in spring, 1/3 in summer and 1/2 at the end of the season. The most favourable influence on the annual grass yield was exercised by the uniform distribution of N. Voisin (1968) provides the theory of N-distribution and explains the practice followed in North-West Europe. In England Carpentier and Louis (1970) examined the effect of 50–50% spring and July distribution and of the use of N in equal doses, respectively, as compared to the spring application of the whole N-dose. In accordance with the treatments they attained seasonally differing yields, the highest at annual level in the case of uniform distribution.

On an irrigated *Alopecurus pratensis*-type hayfield (Bánszki 1973) the distribution of N fertilizer over the vegetation period in rising percentages (25–35–40%) brought a 9% significant yield increase. According to the results of a N distribution experiment carried out in the framework of the COMECON, the uniform distribution during vegetation of larger than 240 kg/ha quantities of N was the most efficient (Bánszki 1974). In the case of grasslands used specially as hayfields, Nagy (1977) suggests concentrating the N-fertilization to the first, or the first two growths.

In the experiment we tried out the most diversified dates and rates of N-distribution in order to determine whether the distribution could be optimized so as to increase the yield, and to what extent the output of each growth could be influenced by different N doses.

Materials and methods

Between 1975 and 1980 the effect of distributing 300 kg/ha N, with 100–200 kg/ha PK active agent as base fertilizer, was studied on a grassland planted at Hajdúszoboszló, on the grass-trial grounds of the Debrecen University of Agricultural Sciences. The treatments are shown in Table 1. The effect of N applied once in autumn, at equal and changing, decreasing and increasing rates during vegetation, and distributed fractionally, was studied as compared to the effect of N supplied only once in spring.

The experiment was laid out in random block design with 4 replications, in plots of 24 m^2 each, with a 4-cutting system. The composition and seed quantities of the grass were: meadow fescue — *Festuca pratensis* HUDS. (Szarvasi-54) 14-, blue grass — *Poa pratensis* L. ssp. *lat.* ("G") 4-, red fescue — *Festuca rubra* L. ssp. *genuina* HACK. ("G") 3-, dactylis — *Dactylis glomerata* L. (Szarvasi-51) 8-, Hungarian brome grass — *Bromus inermis* LEYSS (Szarvasi-52) 6-, timothy — *Phleum pratense* L. ("G") 3-, rye-grass — *Lolium perenne* L. ("G-658") 5-, lódi here — *Trifolium repens* var. *giganteum* LAGR. 2 kg/ha.

The soil of the experiment was a lime-coated lowland chernozem. The results of soil analysis of the 0–20 cm layer prior to starting the experiment are: pH(KCl) 6.2, K_A 44, total salt % 0.02, humus % 3.4, nutrient content in ppm NO_3+NO_2 1.7, AL-soluble P_2O_5 44 and K_2O 239, Mg 575, Na 50, Zn 1.4, Cu 5.3, Mn 100, SO_4 11.7.

The major data of precipitation and temperature in the years of the experiment are contained in Table 2. Apart from 1980 the annual precipitation was less in each year than the 50-year average; 1976 and 1979 being particularly dry years. The differences in the yields of the successive growths were caused by the alternation of dry and wet seasons during the 6th year. The amount of precipitation in the hydrological year is presented, as is a calculation of precipitation per cutting. To the yield of the first growth the late autumn precipitation of the previous year, as well as the winter and spring precipitation, also contributed. To the second growth an average of 108 mm (56–175), to the third growth 112 mm (73–157 mm)

Table 1

Treatments in the experiment
(Base fertilization: PK 100–200 kg/ha active agent)

Treatment (1)	% distribution of N active agent (2)					kg/ha distribution of N active agent (5)				
	autumn (3)	1	2	3	4	autumn (3)	1	2	3	4
		distributed before cutting (4)					distributed before cutting (4)			
1.	—	100	—	—	—	—	300	—	—	—
2.	20	20	20	20	20	60	60	60	60	60
3.	—	25	25	25	25	—	75	75	75	75
4.	50	50	—	—	—	150	150	—	—	—
5.	—	50	—	50	—	—	150	—	150	—
6.	33	33	—	33	—	100	100	—	100	—
7.	—	75	—	25	—	—	225	—	75	—
8.	—	50	25	25	—	—	150	75	75	—
9.	—	50	17	17	17	—	150	50	50	50
10.	—	17	50	17	17	—	50	150	50	50
11.	—	17	17	33	33	—	50	50	100	100
12.	—	—	33	33	33	—	—	100	100	100
13.	—	33	33	17	17	—	100	100	50	50
14.	—	50	50	—	—	—	150	150	—	—

Table 2

Precipitation- and temperature data from the years of the experiment

Years	Amount of precipitation, mm (2)								Mean temperature °C (8)
	Yearly total 1st Jan.— 31st Dec. (3)	Hydrological year 1st Oct.— 30th Sept. (4)	Vegetation period 1st Apr.— 31st Sept. (5)	per cutting (6)					
				1.	2.	3.	4.	Totally (7)	Year's average (9)
1975	541	661	411	110	131	120	41	402	9.7
1976	518	476	330	50	56	126	133	365	8.7
1977	531	537	274	68	38	89	61	256	9.1
1978	573	598	400	112	134	108	38	392	8.4
1979	513	525	270	52	112	73	1	238	9.5
1980	720	631	481	65	175	157	41	438	7.8
Average (10)	566	571	361	76	108	112	52	349	8.9
50 years' average (11)	583	583	340	—	—	—	—	—	10.0

x = Precipitation falling to the first cutting from 1st April (12)

and to the fourth growth 52 mm (1–133 mm) precipitation fell on the average of 6 years. This explains the great differences between the yields of the cuttings.

In the experiment 34% ammonium nitrate was used for N-fertilization, 18% granular superphosphate for P fertilization and 60% KCl for K fertilization. The N-fertilizer was distributed according to the treatments, the P and K fertilizers were supplied only once in autumn.

On the average of the years of the experiment the first cutting was done on 22 May (19–28 May), the second on 4th July (between 27 June and 17 July), the third on 14 August (12–29 August) and the fourth on 21 September (between 16 September and 1 October). The first growth took 68 days from 15th March to develop, the second and third growths 44 days each, while the number of days required for the grass to be ready for the fourth cutting was 39 on the average of the 6 years.

In the major treatments plant samples were taken from each growth of every year in order to determine the components of the grass. The analyses were performed at the Laboratory of the Crop Production Department of the University. The experiment was evaluated with statictical methods and variance analysis. The yields are expressed in terms of absolute dry matter.

Results

Annual yields

The yields were compared to treatment 1 as a relative control (Table 3). The various patterns of N-distribution did not result in yield increase. The different ways of N distribution caused yield differences between the treatments in accordance with the annual and seasonal distribution of precipitation. On the average of 6 years treatments 11 and 12 — which received very small if any doses of N in spring — showed significant yield reduction compared to treatment 1. In several years of the experiment significant differences were found now in one, now in another treatment according to the changes in the annual and seasonal distribution of precipitation, while there were years when no reliable yield differences were obtained owing to the dry weather.

The annual level of yields was limited by the annual amount of precipitation. In the first years of the experiment the yield fluctuation in treatment 1 was 50–124% (see Table 5).

The different rate autumn application of N-fertilizer did not cause yield differences. The pattern of N distribution optimum for the annual yield (opposed to the full does of N supplied in spring) — which would have resulted in yield increase — did not work with the given climate, soil and grass-type hayfield (Fig. 1).

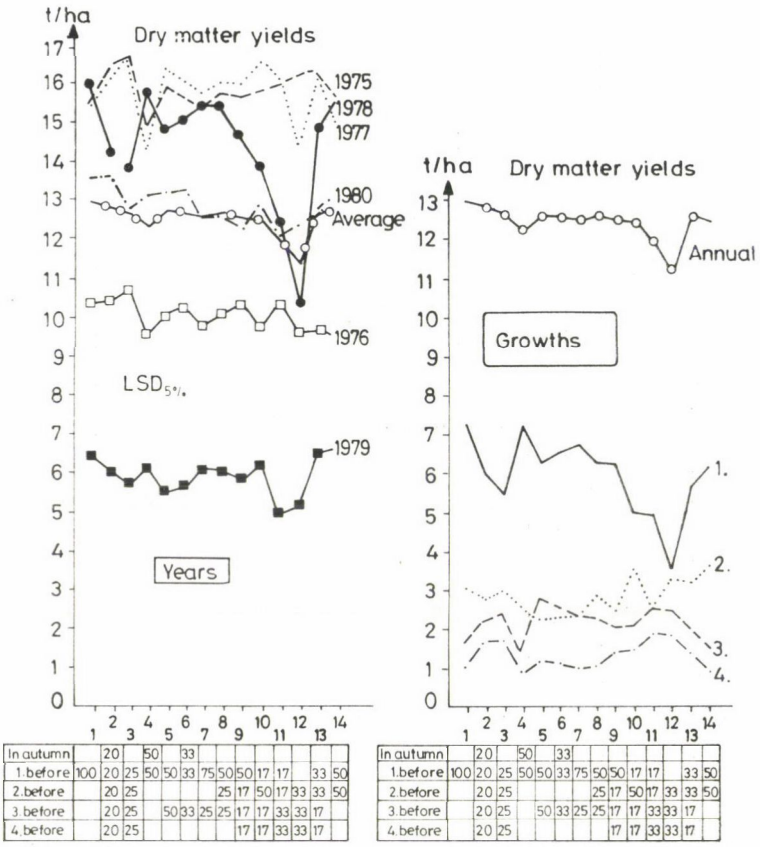
Outputs of cuttings

The 6-year average yield of the growths of grass and its intervals are given in Table 4. The data detailed by cutting show the effect of N-distribution, the yield fluctuations and the so-called year effect (the effect of the amount and distribution of precipitation). With the different cuttings significant difference was obtained now in one, now in another treatment, mainly where the

Table 3

Yield results of the experiment on distributing N-fertilizer in a hayfield
(NPK 300—100—200 kg/ha active agent)

Treatment (1)	Dry matter yield (2)													
	1975		1976		1977		1978		1979		1980		Average (4)	
	t/ha (3)	%	t/ha (3)	%	t/ha (3)	%	t/ha (3)	%	t/ha (3)	%	t/ha (3)	%	t/ha (3)	%
1.	15.58	100	10.35	100	15.49	100	16.07	100	6.46	100	13.63	100	12.93	100
2.	16.50	106	10.46	101	16.21	105	14.26	89	6.04	93	13.65	100	12.85	99
3.	16.64	107	10.72	104	16.68	108	13.87	86	5.71	88	12.78	94	12.73	98
4.	14.86	95	9.56	92	14.33	93	15.78	98	6.20	96	13.15	96	12.31	95
5.	16.00	103	10.16	98	16.45	106	14.82	92	5.53	86	13.27	97	12.71	98
6.	15.77	101	10.27	99	16.15	104	15.08	94	5.64	87	13.30	98	12.70	98
7.	15.39	99	9.78	94	15.77	102	15.46	96	6.18	96	12.59	92	12.53	97
8.	15.80	101	10.18	98	16.11	104	15.49	96	6.11	95	12.60	92	12.72	98
9.	15.73	101	10.34	100	16.03	103	14.67	91	5.87	91	12.34	91	12.50	97
10.	15.86	102	9.72	94	16.57	107	13.79	86	6.24	97	12.80	94	12.50	97
11.	16.01	103	10.40	100	16.15	104	12.40	77	4.98	77	12.22	90	12.03	93
12.	16.26	104	9.62	93	14.48	93	10.27	64	5.19	80	12.39	91	11.37	88
13.	16.28	104	9.67	93	16.12	104	14.79	92	6.52	101	12.83	94	12.70	98
14.	15.66	101	9.39	91	14.94	96	15.73	98	6.74	104	12.90	95	12.56	97
LSD 5%	1.61	10	0.86	8	1.52	10	0.97	6	0.54	8	1.27	9	0.84	6



In autumn		20		50		33									
1. before	100	20	25	50	50	33	75	50	50	17	17		33	50	
2. before		20	25					25	17	50	17	33	33	50	
3. before		20	25		50	33	25	25	17	17	33	33	17		
4. before		20	25						17	17	33	33	17		

In autumn		20		50		33									
1. before	100	20	25	50	50	33	75	50	50	17	17		33	50	
2. before		20	25					25	17	50	17	33	33	50	
3. before		20	25		50	33	25	25	17	17	33	33	17		
4. before		20	25						17	17	33	33	17		

Fig. 1. Effect of various patterns of N-distribution on the yield of the hayfield, 1975–1980

differences of the dose of N were greater. According to the different treatments of N-distribution 3.60–7.27 t/ha yields were obtained with cutting 1, 2.27–3.74 t/ha with cutting 2, 1.50–2.82 t/ha with cutting 3 and 0.87–1.86 t/ha dry matter yield with cutting 4.

Compared to the full dose spring application of N (treatment 1) in the other treatments the outputs of cuttings — except cutting 1 — could be influenced by the different rates and times of N-distribution during the vegetation period, especially in rainier years or seasons.

In Table 5, the yields are detailed by year and growth, and the yearly scatter and fluctuation of the 6-year yield average as well as the proportions of yield level differences are analysed for two selected treatments: treatment 1 (full dose spring application of N) and treatment 3 (uniform distribution of N during the vegetation period). The data show the relationship between yield and precipitation.

Table 4

Yield average and intervals of cutting, 1975—1980

Treatments (1)	Dry matter yield of cuttings, t/ha (2)							
	1.		2.		3.		4.	
	average (3)	interval (4)	average (3)	interval (4)	average (3)	interval (4)	average (3)	interval (4)
1.	7.27	3.86—12.04	3.04	1.32—4.58	1.61	0.47—2.70	1.01	0.21—3.19
2.	6.07	3.09—9.65	2.81	1.06—4.44	2.25	0.55—4.22	1.72	0.15—4.32
3.	5.48	2.55—8.31	3.05	1.13—4.47	2.44	0.52—4.59	1.76	0.24—4.24
4.	7.26	3.92—12.19	2.66	1.05—4.21	1.50	0.47—2.57	0.89	0.16—2.27
5.	6.37	3.16—10.61	2.27	0.97—4.12	2.82	0.58—5.66	1.25	0.19—3.40
6.	6.00	3.21—11.08	2.34	1.04—3.92	2.56	0.63—4.89	1.20	0.23—3.33
7.	6.80	3.48—11.32	2.33	1.11—3.60	2.35	0.69—4.40	1.05	0.23—2.93
8.	6.36	3.06—10.52	2.92	1.26—4.39	2.34	0.55—4.45	1.10	0.17—3.19
9.	6.30	3.03—10.48	2.58	1.09—3.87	2.14	0.57—4.07	1.48	0.19—3.83
10.	5.03	2.28—7.24	3.68	1.12—4.89	2.20	0.56—4.17	1.59	0.21—4.14
11.	4.99	2.04—7.05	2.60	1.05—4.17	2.58	0.61—4.81	1.86	0.22—4.27
12.	3.60	1.60—5.56	3.37	1.17—4.74	2.56	0.59—4.75	1.84	0.22—4.22
13.	5.75	2.88—8.99	3.29	1.07—4.74	2.17	0.66—4.06	1.49	0.19—3.60
14.	6.28	3.04—10.30	3.74	1.24—5.24	1.67	0.47—2.65	0.87	0.17—2.54
LSD 5%	0.90		0.42		0.38		0.35	

Changes in the yield proportions of cuttings in response to the N-distribution are shown in Table 6, as compared to treatment 1. With the first cutting the different N doses resulted in 50–100% yield, with the second cutting in 75–123%, while with the third and fourth cuttings 93–175% yield compared to the full dose N-fertilizer. The figures indicate the possibility of influencing the output of the individual cuttings by the way of distributing the N-fertilizer.

The distribution percentage of the 6-year average yield by cutting changes with the distribution pattern of N: it is 32–56% with the first, 18–32% with the second, 12–22% with the third and 7–16% with the fourth cutting. The yield distribution refers to the total yield (the effect of the natural growth — soil fertility, unfertilized control — and the effect of N-distribution are jointly indicated).

The experiment proved the importance of N-fertilization in spring; furthermore, that the yields of growths can to some extend be influenced by the distribution pattern of N and uniform yields can be ensured by evenly distributed N-doses.

Height of the grass stand

Table 7 contains the data of plant stand height per year and cutting, on the average of 6 years. On year level treatment 10 showed significant differences compared to treatment 1. With the individual cuttings in a number of treat-

Table 5

Yields of cuttings and rates of fluctuation of yield in the experimental years in selected treatments

Number of treatment (1)	Years (2)	Dry matter yield of cuttings (3)								Total (4)	
		1.		2.		3.		4.			
		t/ha (5)	% of aver. (6)	t/ha (5)	% of aver. (6)	t/ha (5)	% of aver. (6)	t/ha (5)	% of aver. (6)	t/ha (5)	% of aver. (6)
1.	1975	7.45	102	4.35	143	2.35	146	1.43	142	15.58	120
	1976	4.05	56	1.32	43	1.79	111	3.19	316	10.35	80
	1977	8.84	122	3.17	104	2.70	168	0.78	77	15.49	120
	1978	12.04	166	2.86	94	0.96	60	0.21	21	16.07	124
	1979	3.86	53	1.92	63	0.47	29	0.21	21	6.46	50
	1980	7.35	101	4.58	151	1.41	88	0.29	29	13.63	105
	Average	7.27	100	3.04	100	1.61	100	1.01	100	12.93	100
3.	1975	6.21	113	4.47	147	3.15	129	2.81	160	16.64	131
	1976	3.15	57	1.13	37	2.20	90	4.24	241	10.72	84
	1977	6.78	124	3.04	100	4.59	188	2.27	129	16.68	131
	1978	8.31	152	2.85	93	2.24	92	0.47	27	13.87	109
	1979	2.55	47	2.40	77	0.52	21	0.24	14	5.71	45
	1980	5.93	108	4.40	144	1.93	79	0.52	30	12.78	100
	Average	5.48	100	3.05	100	2.44	100	1.76	100	12.73	100
Control	1975	2.52	141	1.07	100	2.23	202	1.47	186	7.29	153
	1976	1.09	61	0.79	74	0.85	77	2.03	257	4.76	100
	1977	2.22	124	1.62	151	1.67	152	0.88	111	6.39	135
	1978	2.05	115	0.87	81	0.70	64	0.11	14	3.73	79
	1979	0.74	41	0.96	90	0.26	24	0.09	11	2.05	43
	1980	2.12	118	1.10	103	0.86	78	0.17	22	4.25	89
	Average	1.79	100	1.07	100	1.10	100	0.79	100	4.75	100

ments the differences in height are reliable. The height more or less follows the tendency of yield in the treatments.

N content and yield of the grass

The annual N percentage of the major treatments of the experiment increased in response to the distribution of N, in treatments 3 and 11 significantly compared to treatment 1. In dry years (1976, 1979) the N concentration was higher (Table 8 and Fig. 2).

The content of the growths was lower at the time of the first cutting, while in the further cuttings it was mostly significantly higher owing to the

Table 6

Yield proportions of cuttings and distribution percentages of annual yields in the experiment of distributing N-fertilizer over a hayfield, 1975—1980

Number of treatment (1)	Yield proportions of cuttings in % compared to treatment 1. (2)				Distribution of annual dry matter yield in % per cutting (3)			
	1.	2.	3.	4.	1.	2.	3.	4.
1.	100	100	100	100	56	24	12	8
2.	83	92	140	170	47	22	18	13
3.	75	100	152	174	43	24	19	14
4.	100	88	93	88	59	22	12	7
5.	88	75	175	124	50	18	22	10
6.	91	77	159	119	52	18	20	9
7.	94	77	146	104	54	19	19	8
8.	87	96	145	109	50	23	18	9
9.	87	85	133	147	50	21	17	12
10.	69	121	137	157	40	29	18	13
11.	69	86	160	184	41	32	21	15
12.	50	111	159	182	32	30	22	16
13.	79	108	135	148	45	25	17	12
14.	86	123	104	86	50	30	13	7
LSD 5%	12	14	24	34				

Table 7

Height of growths in the experiment on nitrogen distribution in hayfield, 1975—1980
(NPK 300—100—200 kg/ha active agent)

Treatments (1)	Height of the plant stand, cm (2)				
	Growths (3)				Total (4)
	1.	2.	3.	4.	
1.	77	49	33	26	185
2.	74	46	39	33	192
3.	72	49	39	33	193
4.	79	46	31	23	179
5.	76	40	44	30	190
6.	77	41	43	29	190
7.	78	44	40	26	188
8.	77	48	40	27	192
9.	75	46	37	31	189
10.	70	52	40	32	194
11.	67	45	43	34	189
12.	61	50	43	34	188
13.	73	49	39	32	193
14.	75	52	34	23	184
LSD 5%	3.7	2.6	4.1	4.2	7.8

Table 8

Yearly N contents in the major treatments of the experiment of N-fertilizer distribution in hayfield, 1975—1980

Number of treatment (1)	N content in terms of dry matter percentage (2)													
	1975		1976		1977		1978		1979		1980		Average (4)	
	N % (3)	Rel. %	N % (3)	Rel. %	N % (3)	Rel. %	N % (3)	Rel. %	N % (3)	Rel. %	N % (3)	Rel. %	N % (3)	Rel. %
1.	1.85	100	2.43	100	1.53	100	1.69	100	2.73	100	2.34	100	2.09	100
3.	1.93	104	2.58	106	1.57	103	2.02	120	2.81	103	2.49	106	2.23	107
11.	1.92	104	2.61	107	1.53	100	2.11	125	2.84	104	2.53	108	2.26	108
13.	1.91	103	2.53	104	1.52	99	1.96	116	2.80	103	2.35	100	2.18	104
LSD 5%													0.10	5

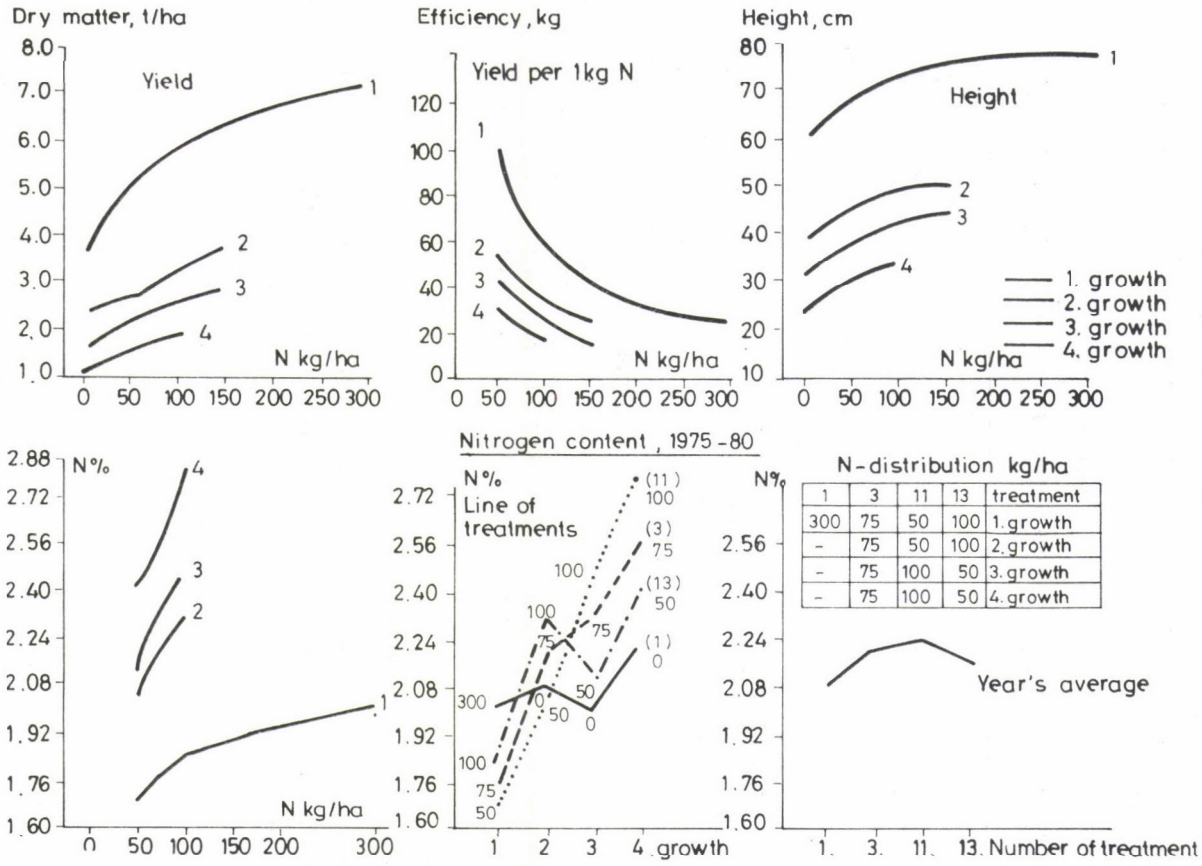


Fig. 2. Effect of various patterns of N-distribution as manifested in the growths and treatments, 1975–1980

distribution of N (Table 9). The per ha N output of the cuttings varied with the treatments, though on an annual level the difference was inconsiderable. The percentage and yield of N with the different cuttings can be influenced by the pattern of N-distribution. The distribution of the yield of N by growth is only slightly different from the yield distribution of the same treatments (see Table 6).

Table 9

N content and yield of cuttings in the major treatments of the experiment of N-fertilizer distribution in hayfield, 1975—1980

Number of treatment (1)	Cuttings (2)									
	1.		2.		3.		4.		Average/Total (4)	
	(3)	%	(3)	%	(3)	%	(3)	%	(3)	%
	N content in terms of dry matter percentage (5)									
1.	2.08	100	2.08	100	2.00	100	2.22	100	2.09	100
3.	1.80	87	2.22	107	2.36	118	2.56	115	2.23	107
11.	1.74	84	2.06	99	2.48	124	2.75	124	2.26	108
13.	1.85	89	2.35	113	2.15	108	2.36	106	2.18	104
LSD 5%	0.09	4	0.11	5	0.12	6	0.22	10	0.10	5
	N yield kg/ha and its distribution % (6)									
1.	151.2	56	63.2	24	32.2	12	22.4	8	269.0	100
3.	98.6	37	67.7	25	57.6	21	45.1	17	269.0	100
11.	86.8	34	53.6	21	64.0	25	51.2	20	255.6	95
13.	106.4	40	77.3	29	46.7	18	35.2	13	265.6	99

Efficiency of N distribution

On the average of several years, the N-distribution did not show differences in efficiency between the treatments owing to the long term and after-effect of the N-fertilizer distributed every year over the successive growths. The same dose of N used up by the growths resulted in different yield distributions. The efficiency of the same N-dose distributed during the vegetation period decreases (Table 10 and Fig. 2). The tendency of yield per 1 kg N decreases with the increase of the N dose and in the later growths. The yield per 1 cm grass height increases in response to rising doses of N, but decreases with the successive cuttings.

The 100% spring application of the N-fertilizer was the most economical in the experiment; this treatment avoids the working peaks, reduces the demand of machine capacity, but the distribution of yield is unfavourable. The method of N-distribution had better be decided with the purpose of production, the annual schedule of harvesting as well as economic calculations and

Table 10

Efficiency of the quantity of N used in the experiment of N-fertilizer distribution in hayfield, as manifested in the cuttings, 1975—1980
(NPK 300—100—200 kg/ha active agent)

N used up in the cuttings N kg/ha (1)	Distribution of yield, % (2)				Yield per 1 kg N fertilizer (4)				Yield per 1 cm grass height, kg (5)			
	1.	2.	2.	4.	1.	2.	3.	4.	1.	2.	3.	4.
	cutting (3)				cutting (3)				cutting (3)			
50	40	21	17	12	100	52	43	31	73	57	56	49
60	—	22	18	13	—	47	38	29	—	61	58	51
75	43	24	19	14	73	40	32	23	76	63	59	53
100	45	26	21	16	58	33	26	19	78	67	70	55
120	47	—	—	—	51	—	—	—	82	—	—	—
150	50	29	22	—	42	25	19	—	82	—	—	—
200	52				33				86			
225	54				30				87			
300	56				24				93			

precipitation conditions taken into consideration. On this basis the distribution of N for the individual growths can be optimized.

Composition of the grass stand

The distribution of identical annual amounts of N did not result in composition differences between the treatments.

Summary

Between 1975 and 1980 in a planted grassland used as hayfield, various distribution patterns of 300 kg/ha N (applied in autumn, in spring, in uniform-, changing-, decreasing-, increasing- and fractional doses) was studied in comparison to the 100% spring application. The soil of the experiment was a lime-coated lowland chernozem.

On the average of 6 years the treatments did not result in yield increases and yield reductions were obtained when small quantities of N or none were used (treatments 11 and 12). In the years of the experiment the yield level was determined by the annual amount and distribution of precipitation. Rainy and dry years and seasons alternated. Under the conditions of the experiment an optimum N-distribution could not be established. The yields of the different growths also showed variation as a function of precipitation distribution and N dose. The outputs of cuttings could be influenced by the rate N-fof ertilization. The distribution of the N-dose over the vegetation period increased the nitrogen content of the grass. The different N doses were utilized in the cuttings in different ways.

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 397–407 (1991)

THE EFFECT OF MOWING-FREQUENCY, N-QUANTITY AND WATERING ON IRRIGATED STANDS

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(Received: 21 September, 1990; accepted: 21 December, 1990)

We have studied the effect of 3 and 6 mowings, and of N 150 and 300 kg/ha/year on dry and on watered stands, between mowings on 25th April and 25th September. The basic amount of P_2O_5 50, K_2O fertilizers used was 100 kg/ha/year.

The increase of number of mowings reduced dry matter yields by 29–43%; higher N-doses meant a 17–32% increase, while watering raised yields by 35–60%. According to regression analysis the yield was determined in the following ratio: number of mowings 53%, way of cultivation 36% and N-fertilization 11%. When mowing was carried out 6 times, the mineral content of the stand was higher. The number of mowings played a decisive role in the mineral content, determining it in 58–95%. The increase of N-fertilization augmented while that of watering reduced the concentration of different elements. The abundance ratio of plant species also changed.

Keywords: grassland, mowing-frequency, N-quantity, watering

Introduction

The increase of the number of mowings and of N-doses, as well as watering alter the size of yields, the element content and the ratio of different plant species in the stands (4, 8). The trends and degree of changes differ lawn by lawn. The increase of the number of mowings resulted in yield reduction in most of the experiments (1, 2, 8) in some cases it increased the dry-matter yield (9) and differences were reduced by growing N-doses (3).

The increase of the numbers of mowings, and of N-doses, also increased the protein-contents and yield, as well as the mineral content for each case studied (1, 6, 8). Parallel to mowing-frequency the P, Ca and Mg content decreased due to high N-doses, while N and K concentrations increased (11). Less frequent mowing was favourable for meadow fescue (5) and for meadow foxtail (10) as shown by their increased ratios among grass-species. More frequent mowing increased the rate of blue-grass (1).

Materials and methods

Between 1987 and 1989 we studied the effect of 3 and 6 mowings (with 60 and 30 days of regeneration, respectively), and that of N 150 and 300 kg/ha/year on dry and watered stands in Debrecen, between 25th April and 25th September. The uniform amount of basic P_2O_5 50, K_2O fertilization was 100 kg/ha/year over the whole experiment.

The experiment was made in four parallels, in random block-design on plots of 28 m². The soil in the experiment was chernozem of the plain-type, with lime-precipitation. Before starting the experiment the result of soil-analyses of the 0–20 cm layer were: pH (KCl) 7.3–7.5, soil-hardness according to Arany 36–37, total salt percentage 0.02, $CaCO_3$ % 6–10, humus % 1.4–1.6; nutrient supply in ppm: $NO_3 + NO_2$ 4–6. Al-soluble P_2O_5 921–1190, K_2O 243–270, Mg 131–140, Zn 3–5 and Cu 3–4. The soil in the experiment was supplied well with phosphorus and potassium and at a medium degree with humus, according to the categories of MÉM NAK (Hungarian Centres of Agrochemistry). The area was flat land, 100 m above sea-level, and the water-plane was approximately 3 m below the surface.

In the experiment, 34% ammonium-nitrate was used as N-fertilizer, distributed in 3 and 6 equal doses. The first dose was dispersed on 25th March, then the other doses followed on the day after each mowing, according to the mowing schedule of 30 and 60 days. P-fertilization was made with 18% grained superphosphate, while 60% KCl was used for K-fertilization, both in a single dose in autumn. In the years of the experiment the annual precipitations were 590, 541 and 576 mm, being 303, 304 and 434 in the vegetational period. The annual mean temperature values were 8.7, 8.6 and 9.1 °C (averages for 50 years were: 583 mm, 340 mm and 10.0 °C).

In watered plots sprinkling was applied 9 times a year with doses of 40 mm, altogether a total 360 mm of water.

The stands were planted at the beginning of Summer, 1985, and the experiments were set up in Autumn, 1986. The species composition of the stand and the amount of seeds used were as follows: *Festuca pratensis* HUDS. — Szarvasi-54 12, *Poa pratensis* L. ssp. lat. — Szarvasi-59 9, *Festuca rubra* L. ssp. genuina HACK — Szarvasi-58 5, *Dyctylis glomerata* L. — Szarvasi-51 3, *Bromus inermis* LEYSS — Szarvasi-52 4, *Trifolium repens* L. — Lovászpatonai-4 *Lotus corniculatus* L. — "G" 4 kg/ha.

The results of the botanical survey were given as abundance percentages. The yield was measured in green form; the dry matter content was determined after the samples dried, and was expressed as the absolute value of the dry matter content. For the determination of macro- and microelements samples of 2 kg were taken at each mowing every year, using the average samples of treatments and parallels. The analyses were carried out according to the methods of MÉM NAK, using an ICP equipment, in the Central Laboratory of the University of Agricultural Sciences of Debrecen. On the basis of Laboratory data, weighted averages were calculated.

The results were evaluated using statistical methods, analysis of variance and multivariate regression (Mundruczó 1981).

Results and discussion

Dry-matter yield, effectiveness

On the basis of 3 years' averages (Table 1) the increase of mowing frequency from 3 to 6 per year reduced the dry matter yield — at different N-levels — by 39–43% in dry stands and by 29–36% in watered stands.

The N 300 dose increased the yield by 17–24% in dry stands and by 19–32% in watered ones, as compared to the N 150 dose, with different mowing frequencies.

Watering, together with 3 mowings, increased yields by 35–38% and with 5 mowings by 50–60% at N-levels on the average of 3 years.

According to regression analyses (Table 6) the dry-matter yield was determined in the following ratio: by N-fertilizer in 11%, by number of mowings in 53%, by cultivation-type (watering) in 36%, according to the following equation:

$$y = 10.41 + 0.017x_1 - 1.849x_2 + 4.538x_3, \qquad R = 0.99$$

Table 1

The effect of mowing frequency, N-dose, dry- and watered cultivation on the dry matter yield, on effectivity indices and on plant height. Debrecen, 1987–89
(Basic fertilization: P_2O_5 50 and K_2O 100 kg/ha/year)

Cultivation type	Dry				
Number of mowings	3		6		LSD 5%
N kg/ha/year	150	300	150	300	
Dry-matter yield					
t/ha	12.8	14.10	6.94	8.60	3.83
%	100	117	57	71	31
Effect %					
Mowing	100	100	57	61	
N-dose	100	117	100	124	
Watering	100	100	100	100	
Effectiveness indices					
Yield per 1 kg of NPK	40	31	23	19	
NPK substance per 1 t of yield	25	32	43	52	
Height of plant stand					
Annual, cumulated height, cm	131	153	93	114	39
	100	117	71	87	30

Cultivation type	Watered				
Number of mowings	3		6		LSD 5%
N kg/ha/year	150	300	150	300	
Dry-matter yield					
t/ha	16.29	19.42	10.41	13.75	3.57
%	100	119	64	84	22
Effect %					
Mowing	100	100	64	71	
N-dose	100	119	100	132	
Watering	135	138	150	160	
Effectiveness indices					
Yield per 1 kg of NPK	54	43	35	31	
NPK substance per 1 t of yield	18	23	29	33	
Height of plant stand					
Annual, cumulated height, cm	166	192	125	159	39
	100	116	75	96	23

As shown by indices of effectiveness the most effective treatments were, for the total yield: 3 times of mowing and N 150 kg/ha combined by either dry or watered cultivation, since the yields per 1 kg NPK fertilizer were 40 kg and 54 kg in dry and watered stands, respectively. The amount of NPK mixed substance used for 1 t of yield is also the most favourable in this combination of treatments, being 25 kg for dry stands and 18 kg for watered ones.

The distribution of dry-matter yields for each mowing and their seasonal changes are also shown in detail (Table 2). The difference between dry-matter yields of dry and watered stands was lower in spring and early summer, in favour of watered stands (being 3–23% and 32–33%, respectively). In green

Table 2

The effect of mowing frequency, N-dose, dry- and watered cultivation on the distribution of dry-matter yield of stands among mowings
Debrecen, 1987–89
(Basic fertilization: P_2O_5 50 and K_2O 100 kg/ha/year)

Number of mowings	N kg/ha/year	Date and dry-matter yield at each mowing (t/ha)					
		IV. 25.	V. 25.	VI. 25.	VII. 25.	VIII. 25.	IX. 25.
				Dry			
3	150	—	6.89	—	2.51	—	2.68
3	300	—	7.91	—	3.23	—	2.96
6	150	0.99	2.48	1.49	0.32	0.55	1.11
6	300	1.45	2.79	1.86	0.39	0.67	1.44
				Watered			
3	150	—	6.96	—	4.68	—	4.65
3	300	—	8.46	—	5.51	—	5.45
6	150	1.02	2.86	1.98	1.46	1.57	1.52
6	300	1.56	3.44	2.54	2.09	2.22	1.90
		Effect of watering on each mowing, % (Dry = 100%)					
3	150	—	101	—	186	—	174
3	300	—	107	—	171	—	184
6	150	103	115	132	456	285	137
6	300	108	123	137	536	331	132

crop the differences were higher. In the summer period the yield-increasing effect of watering was more expressed, resulting in the multifold increase of yields in July–August, being 71–436%. In September this effect was already moderate (32–84%). The results show indirectly which is the period when it is worthwhile and useful to water the stands, in order to increase the yield and to make it more seasonally uniform.

We have also calculated the distribution of yield in each mowing. A more uniform distribution was obtained in watered stands (between 10 and

44%) than in dry ones (4–57%). With a frequency of 3 mowings the yield distribution was 21–57% in dry stands and 28–44% in watered ones. With a 6-mowings' frequency there was a peak yield in May, being more than half of the annual yield in dry stands (56–57%) and somewhat less in watered ones (43–44%).

Plant-height in the stands

The height of the grass stand varied between 93 and 153 in dry ones and 125–192 cm in watered ones (annual, cumulated value, Table 1). Plant height was greater with 3 mowings, being 131–153 for dry stands and 166–192 for watered ones, at different N-levels. With 6 mowings heights were 93–114 cm in dry stands and 125–159 in watered ones. The height-differences of N-levels are not reliable, while being at the border of significance among mowing frequencies. According to regression analysis (Table 6) height was determined — on an average of the experiments — equally by mowing frequency and by cultivation-type at a 41–41% ratio, while the role played by the N-fertilizer was 18%.

The element-content and -yield of the stand

With the mowing-frequency of 6 the element-content of the stand was higher than with 3 mowings (by 23–57% relatively), the differences being significant for elements N, P, K and Ca (Table 3). The N, Ca, Mg and Cu contents of the watered stand was lower (by 3–26% relatively), but the concentrations of P, K and Zn were relatively higher than those of dry stands. The 300 kg/ha dose of N increased the N, K, Mg, Zn and Cu-contents, as compared to the N 150 dose (by 6–25% relatively), while decreasing the P and Ca percentages for both dry and watered stands (by 5–10% relatively); the difference is reliable in the case of the N-content.

The (weighted) N-content varied between 1.62–2.82% for dry stands and between 1.27 and 2.31% for watered ones, depending on the N-dose and on mowing frequency. The N-percentage was 1.27–2.03 with 3 mowings, while being 2.00–2.84% with 6 mowings in dry and watered stands at different N-levels. When a N-dose of 150 kg/ha was applied, the N-content of both dry and watered stands varied between 1.27 and 2.51% for different mowing-frequencies, while the range was 1.50 to 2.84% with the N-dose of 300 kg/ha. The contents of other elements also varied according to a peculiar trend. The range of P-content was 0.20–0.35%, that of the K-content was 2.15–3.24%, for Ca-content it was 0.38–0.65% and for Mg-content it was 0.13–0.24%. In various treatments the Zn-content varied between 14.1–27.7 ppm and the Cu-content was 6.1–10.8 ppm.

Table 3

The effect of mowing frequency, N-dose, dry- and watered cultivation on the element-content of the

Cultivation type	Dry			
Number of mowings	3		6	
N kg/ha/year	150	300	150	300
	Element content, as % of dry matter (weighted)			
N %	1,62	2.03	2.51	2.84
P %	0.21	0.20	0.30	0.28
K %	2.15	2.27	2.67	2.84
Ca %	0.45	0.42	0.65	0.62
Mg %	0.17	0.18	0.21	0.24
Zn ppm	14.1	15.8	21.2	22.9
Cu ppm	8.0	8.7	10.1	10.8
			Yields of macroelements, kg/ha	
N-yield	195.7	285.9	173.9	244.2
P-yield	25.7	28.3	20.5	24.5
K-yield	260.1	320.2	185.0	244.4
Ca-yield	54.2	59.6	45.2	53.1
Mg-yield	20.0	25.6	14.8	20.7

As shown by regression-analyses (Table 6) the contents of different elements were determined by mowing frequency at a 58–95% ratio, by cultivation-type at a 2–28% ratio, while the contribution of N-fertilizer was 3–10%.

The yields of macroelements per hectare were calculated on the basis of yield and of weighted element-contents. The trends of yields of elements differed from the contents of elements. At identical N-levels, when 3 mowings were made, the yield of elements was higher than with 6 mowings, except the N-yield of watered stands at both N-levels and Ca-yield at N 300. The yield of elements of the watered stand per ha is usually higher than that of dry stands, sometimes even despite the decreased specific content of the given element. The N-dose of 300 resulted in higher yields of elements both in dry and in watered stands.

Table 4 shows the element content for each mowing in the vegetational period. The trends obtained for the annual surveys are valid here as well, but the detailed data have some further peculiarities. The N, Ca, Mg, Zn and Cu-contents showed a tendency to increase from spring till autumn, in both dry and watered stands, with every mowing-frequency and N-dose; the values of P and K decreased with dry cultivation, but increased with watering. The element-contents, either increasing or decreasing, were generally more uniform when watering was applied.

stand and on its yields of macroelements per ha Debrecen, 1987–89

LSD 5%	Watered				LSD 5%
	3		6		
	150	300	150	300	
0.21	1.27	1.50	2.00	2.31	0.10
0.04	0.27	0.25	0.35	0.33	0.04
0.26	2.44	2.65	3.01	3.24	0.20
0.10	0.42	0.38	0.63	0.59	0.09
0.07	0.13	0.15	0.17	0.21	0.03
2.9	18.2	19.5	24.9	27.7	4.8
1.4	6.1	6.9	8.8	9.6	1.3
	207.2	291.2	208.1	317.5	
	43.8	48.5	36.8	45.3	
	397.6	515.5	313.5	445.5	
	68.2	73.8	65.9	81.5	
	21.9	29.4	18.2	28.9	

The structure and species composition of the stand

We evaluated the abundance percentages at the end of the experiment. By increasing the frequency of mowing from 3 to 6 the ratio of grass species decreased in both the dry and the watered stands (Table 5). Legumes could not compete in the stands as a result of higher N-dose and mowing frequency, even in the watering experiment.

The changes of the structure of stands were definitely influenced by the mowing-frequency, in 87–95% according to regression-analysis (Table 6).

The abudance of the important grass species also varied. When mowing was carried out 3 times, the ratio of *Poa pratensis* decreased both in dry and in watered stands; the percentage of *Dactylis glomerata* in watered stands and the frequency of *Bromus inermis* in dry ones increased. The higher N-dose decreased the ratio of *Poa pratensis* in both dry and watered stands, while increasing the ratio of *Bromus inermis* in the dry stand. The abudance percentage of *Festuca pratensis* decreased by the end of the experiment in both the dry and the watered experiment. According to the results of regression analysis (Table 6), the ratio of *Poa pratensis* was primarily determined by the mowing-frequency, while those of *Dactylis glomerata* and *Bromus inermis* were determined mostly by the cultivation methods, on the experimental average.

Table 4

The effect of mowing frequency, N-dose, dry- and watered cultivation on the content of the main elements in the stand at each mowing
Debrecen, 1987–89

Cultivation type		Dry				Watered			
Number of mowings		3		6		3		6	
N kg/ha/year		150	300	150	300	150	300	150	300
Elements	Date	Element content, as % of dry matter							
N %	IV. 25.			2.7	3.0			2.6	2.7
	V. 25.	1.4	1.9	2.3	2.6	1.1	1.5	1.6	2.1
	VI. 25.			2.3	2.6			1.8	2.0
	VII. 25.	1.6	2.0	2.6	2.8	1.3	1.4	2.2	2.3
	VIII. 25.			3.0	3.3			2.1	2.3
	IX. 25.	2.2	2.4	2.8	3.2	1.5	1.6	2.5	2.8
P %	IV. 25.			0.33	0.32			0.34	0.33
	V. 25.	0.21	0.21	0.30	0.29	0.24	0.23	0.32	0.32
	VI. 25.			0.28	0.27			0.34	0.27
	VII. 25.	0.18	0.16	0.25	0.25	0.28	0.26	0.36	0.32
	VIII. 25.			0.27	0.25			0.40	0.37
	IX. 25.	0.25	0.22	0.30	0.28	0.30	0.27	0.42	0.39
K %	IV. 25.			2.94	3.05			2.97	3.14
	V. 25.	2.19	2.20	2.77	2.83	2.47	2.62	2.88	3.15
	VI. 25.			2.71	3.05			3.03	3.14
	VII. 25.	2.10	2.52	2.40	2.48	2.25	2.78	3.03	3.21
	VIII. 25.			2.36	2.57			3.34	3.52
	IX. 25.	2.11	2.19	2.35	2.55	2.59	2.58	3.25	3.32
Ca %	IV. 25.			0.56	0.49			0.51	0.46
	V. 25.	0.33	0.32	0.48	0.44	0.29	0.29	0.44	0.41
	VI. 25.			0.66	0.62			0.61	0.55
	VII. 25.	0.56	0.52	0.77	0.70	0.49	0.43	0.68	0.61
	VIII. 25.			0.77	0.75			0.73	0.69
	IX. 25.	0.65	0.59	1.02	0.99	0.54	0.47	1.01	0.96
Mg %	IV. 25.			0.16	0.17			0.15	0.16
	V. 25.	0.12	0.14	0.17	0.19	0.10	0.12	0.13	0.19
	VI. 25.			0.22	0.24			0.19	0.21
	VII. 25.	0.22	0.23	0.29	0.31	0.18	0.19	0.21	0.22
	VIII. 25.			0.30	0.33			0.18	0.22
	IX. 25.	0.23	0.24	0.28	0.35	0.14	0.16	0.23	0.27
Zn ppm	IV. 25.			24.1	22.8			31.2	31.4
	V. 25.	11.5	13.7	15.9	18.9	15.9	17.1	21.1	21.0
	VI. 25.			24.8	24.6			27.2	29.0
	VII. 25.	14.7	15.5	20.1	20.1	17.9	16.1	24.4	27.2
	VIII. 25.			21.2	22.6			25.6	30.0
	IX. 25.	20.3	21.7	25.7	29.1	21.9	26.8	27.2	32.8
Cu ppm	IV. 25.			10.8	11.1			10.7	10.8
	V. 25.	8.0	8.5	9.2	9.8	5.3	6.4	8.4	9.0
	VI. 25.			9.3	9.5			8.2	8.5
	VII. 25.	6.5	8.0	9.4	9.6	5.9	6.8	8.9	9.1
	VIII. 25.			9.8	10.1			8.8	9.7
	IX. 25.	9.5	9.9	12.9	14.7	7.4	7.9	10.0	11.6

Table 5

The effect of mowing frequency, N-dose, dry- and watered cultivation on the structure of the stand and on the ratios of main grass species at the end of the experiment
Debrecen, 1989. IX. 25.

Cultivation type	Dry					Watered				
Number of mowings	3		6		LSD 5%	3		6		LSD 5%
N kg/ha/year	150	300	150	300		150	300	150	300	
	Abundance percentages									
	Structure of the stand, %									
Grasses	87	88	67	63	10	87	88	78	77	4
Legumes	1	—	—	—	—	2	—	—	—	—
Weeds	5	3	30	32	10	3	3	19	20	4
Nude area	7	9	3	5	1	8	9	4	3	1
	Ratios of the main grass species, %									
Poa pratensis	4	2	32	24	6	6	3	46	47	5
Dactylis glomerata	20	10	29	29	6	75	78	23	23	10
Bromus inermis	61	74	4	8	4	4	5	4	2	1
Festuca pratensis	2	2	2	2	—	2	2	5	5	1

Conclusions

(1) The application of 3 mowings per year, as compared to 6 mowings, increased the dry-matter yield, the yields of elements per ha, but reduced the element content of the stand.

(2) The N-dose of 300 kg/ha increased the yield, the element-yield and the contents of N, K, Mg, Zn and Cu, but reduced the percentages of P and Ca, as compared to 150.

(3) Watering, as compared to dry cultivation, increased yield, the yields of elements, the concentration of P, K and Zn in the grass, but decreased the percentages of N, Ca, Mg and Cu.

(4) The mowing frequency played the decisive role in determining the amount of yield, the contents and yields of minerals in the grass and the ratios of different plant species.

(5) The yield and the element-content of the stand has a seasonal variation. The contents of N, Ca, Mg, Zn and Cu increased from spring till autumn, while the concentrations of P and K decreased in the dry stand and increased in the watered one. Watering made the mineral-content of the stand more uniform.

Table 6

Results of regression analysis
Debrecen, 1987–89

Dependent variables	Equations and correlation values of multivariate regression					The ratios of independent variables determined on the basis of multi-determinant coefficients		
	y = a	± N-fertilizer X_1	± Number of mowings X_2	± Cultivation type X_3	R =	N-fertilizer x_1	Number of mowings x_2	Cultivation type x_3
Dry-matter yield t/ha	$y = 10.41$	$+0.017x_1$	$-1.849x_2$	$+4.538x_3$	0.99	11	53	36
Plant height cm	$y = 103.0$	$+0.17x_1$	$-12.58x_2$	$+37.75x_3$	0.99	18	41	41
Element content								
N %	$y = 1.04$	$+0.002x_1$	$+0.27x_2$	$-0.48x_3$	0.99	10	67	23
P %	$y = 0.098$	$-0.0001x_1$	$+0.028x_2$	$+0.053x_3$	0.99	3	69	28
K %	$y = 1.01$	$+0.001x_1$	$+0.188x_2$	$+0.353x_3$	0.99	7	67	26
Ca %	$y = 0.31$	$-0.0002x_1$	$+0.068x_2$	$-0.03x_3$	0.99	3	95	2
Mg %	$y = 0.12$	$+0.0002x_1$	$+0.012x_2$	$-0.035x_3$	0.99	14	58	28
Zn ppm	$y = 0.70$	$+0.013x_1$	$+2.43x_2$	$+4.08x_3$	0.99	5	72	23
Cu ppm	$y = 6.22$	$+0.005x_1$	$+0.80x_2$	$-1.55x_3$	0.99	6	66	28
Structure of the stand and main grass species, %								
Grasses	$y = 95.5$	$-0.005x_1$	$-5.42x_2$	$+6.23x_3$	0.94	0	87	13
Weeds	$y = -9.25$	$+0.0017x_1$	$+7.25x_2$	$-6.25x_3$	0.97	0	92	8
Nude area	$y = 11.25$	$+0.0067x_1$	$-1.5x_2$	$-0.0x_3$	0.96	5	95	0
Poa pratensis	$y = -40.25$	$-0.02x_1$	$+11.17x_2$	$+10.0x_3$	0.97	1	91	8
Dactylis glomerata	$y = 26.5$	$-0.012x_1$	$-6.58x_2$	$+27.8x_3$	0.71	0	34	66
Bromus inermis	$y = 111.0$	$+0.027x_1$	$-10.50x_2$	$-33.0x_3$	0.83	1	47	52

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 409–416 (1991)

ESTIMATION OF OPTIMUM PLOT SIZE AND SHAPE FOR WHEAT (*TRITICUM VULGARE* L.) YIELD EXPERIMENTS

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(Received: 26 March, 1990; accepted: 15June, 1990)

Data from a uniformity trial on wheat were collected and analysed from 1152 basic unit plots in each of 3 seasons. Each basic unit was 4 m long and 1 m wide. Estimates of optimum size of plots were obtained for the 3 seasons using average costs and individual values of Smith's soil heterogeneity index. These were 14.8, 18.3 and 7.6 basic units with an average value of 13.6 basic units (54.4 m²) for unguarded plots. For guarded plots the estimates were 12.1, 15.0 and 6.2 basic units, with an average value of 11.1 basic units (44.4 m²).

The shapes of the plots were not significantly different but, in general, long narrow plots were less variable. For blocks of all sizes, plots should be placed side by side so as to have compact blocks.

Keywords: heterogeneity index, plot size, uniformity trial

Introduction

In Sudan, sorghum and wheat are the two main stample crops. Wheat is grown excessively in the central plains where the Gezira Research Station is situated. There is a high demand for experimentation on all agronomic and breeding aspects of the crop. In order to carry out these experiments efficiently, the optimum plot size and shape should be determined. This uniformity trial was conducted to achieve this goal.

Smith (1938) developed an empirical law to relate soil heterogeneity to plot size and shape. This law relates V_x the variance among plots X basic units to V_1, the variance among plots of one basic unit, via b, a soil heterogeneity index, viz:

$$V_x = \frac{V_1}{X^b} \tag{1}$$

where $0 < b < 1$.

The value of b is estimated by regressing log V_x on log X. Smith suggested a simple weighting of variances by their respective degrees of freedom. Several other computational procedures were proposed. Koch and Rigney (1951) used experimental data to estimate b instead of uniformity data. Hatheway and

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Williams (1958) suggested a weighted least estimate for Smith's b. Federrer (1955) developed a weighted estimate where the weights were elements inversely proportional to the variances. Pearce (1955) proposed the inclusion in Smith's model of a term, independent of the characteristics of the field, to account for the variation between plants.

Modjeska and Rawling (1983) indicated that the fundamental problem in determining plot size in field trials was that spatially associated experimental units tend to give correlated responses in plant growth due to their share of microenvironmental factors. They argued that because of this positive correlation it was less efficient, as far as treatment comparisons were concerned, to increase plot size by a given number X units than to use an equal number of independent units and that X^b in Smith's model could be regarded as the effective number of independent units in a plot of size X.

For the purpose of this paper, the value of b was calculated by using Smith's weighted analysis, where the weights were the number of degrees of freedom associated with each variance. Then the optimum plot size, X, defined as that area for which the cost per unit of information is minimum (Zuhike and Gritton 1969), was calculated for unguarded plots from the equation:

$$X = \frac{b\,k_1}{(1-b)\,k_2} \tag{2}$$

while that for guarded plots was calculated from the equation:

$$X = \frac{b\,(k_1 + k_g\,A)}{(1-b)\,(K_2 + K_g\,B)} \tag{3}$$

where:

b = the coefficient of soil heterogeneity,
k_1 = the cost per plot,
k_2 = the cost per unit area of test area,
k_g = the cost per unit area of test area,
A = end guard area,
B = ratio of side guards to test area.

Materials and methods

In this experiment wheat was grown in an area of 2.5 feddans (2.585 acres) at the Gezira Research Farm, Sudan. In performing the agricultural operations, the Gezira Station standard field practise was followed. This experiment was repeated for 3 seasons. The repetition had these purposes:

(a) to cover a large area of the farm,
(b) to get a reasonable mean estimate of the costs involved, and
(c) to account for the climatic effects.

At harvest the guards were removed leaving a net area of 96 metres long × 48 metres wide. These were divided into basic unit areas of 4 m × 1 m wide, giving 1152 basic units. Each unit was harvested separately and the produce placed into large sacks which were tagged. Then the heads were threshed and grain yield for each basic unit was weighted and recorded separately. These were combined to give the yields for different shapes and sizes of plots.

In order to calculate the yield in the case of guarded plots, for each shape and size considered, 4 meters between plots end to end and 0.5 m at each side of the plots were discarded.

For both guarded and unguarded plots, the variances among plots of X basic units, $V(x)$, were computed in the usual way, while the variance per basic unit area, V_x, was calculated from the equation:

$$V_x = \frac{V(x)}{X^2}.$$

The comparable variance V was calculated as:

$$V = \frac{V(x)}{X}.$$

The coefficient of variation, $C.V.$, was given as:

$$C.V. = \frac{100\sqrt{V}}{\text{mean (on a per unit basis)}}$$

The coefficient of soil heterogeneity, b, was calculated and the differences between plot shapes having the same number of basic units were tested for significance by comparing their comparable variance, V, applying Bartlett's Chi-square test of heterogeneity.

Results and discussion

Table 1 shows the expressions of yield variation for various plot sizes and shapes for both guarded and unguarded plots for the third season, whereas Tables 2 and 3 show the coefficient of variation for unguarded and guarded plots respectively. From these tables, it is generally true that the variance per basic unit area as well as the coefficient of variation are lowest for long, narrow plots and highest for short, wide plots. The coefficient of variation for unguarded plots ranged from 24.7 for a plot of one basic unit in size, to 12.3 for plot size of 48 basic units. For guarded plots it ranged from 23.9 for a plot of one basic unit, to 12.6 for a plot of 40 basic units in size. This would indicate that the direction of greatest soil variation was parallel to the length of plots, Keller (1949). However, this indication was confirmed by examining a moving average for the yields.

Values of variance per basic unit areas were used to estimate b. The estimate for the third season was 0.40. For the first and second seasons similar results as shown above were obtained but were not shown here for the sake of space saving. However, the estimates of b were 0.56 and 0.62 for the first and second seasons respectively.

The average costs for the 3 seasons were:

k_1 = Ls 0.083 (*Ls* 1 = *S* 1.200 approx.), per plot,

k_2 = Ls 0.007 per sq. m. and

k_g = Ls 0.011.

Thus using equation (2), the optimum size for unguarded plots for the 3 seasons were 14.8, 18.3 and 7.6 basic units respectively with an average value of 13.6 basic units.

As for guarded plots $A = 8$ sq. m. and $B = 1.00$, therefore, using equation (3), the optimum plot sizes for the 3 seasons in succession were 12.1, 15.0 and 6.2 basic units with an average value of 11.1 basic units.

These discrepancies between the optimum plot sizes are due to the difference between the soil heterogeneity values for first season and those of the second and third seasons. The trial was conducted in the southern part of the farm in the first and second seasons and in the northern part during the third season. So, these differences are a reflection of the fertility pattern in these sites of the research farm. However, Wiedemann and Leininger (1963) showed that the range for b from 0.4 to 0.7 would indicate a soil of average uniformity. This description appears to suit our case.

The variation within qlocks of different shapes and sizes has been examined for blocks consisting of 4, 8 and 16 plots. The comparisons, Table 4, are given in terms of the standard error of a treatment mean.

From this table it is clear that, irrespective of the arrangement of plots, small plots have smaller standard errors than do large ones. It might be advisable to avoid arranging the plots end to end within blocks, so that the blocks become long and narrow. The arrangement of single plots or pairs of plots side by side seems to be reasonable. Hodnett (1952), working with groundnuts, reached the same conclusion.

The comparable variances of each size, for all possible shapes, showed no significance, when tested for heterogeneity. The main reason for the decrease in variance could be considered as a result of the increased size of plots. Thus, it appears that the estimated optimum size of 13.6 basic units (54.4 m^2) for unguarded plots and 11.1 basic units (44.4 m^2), test area, for guarded plots, can be arranged in any convenient way.

The pattern of variability for unguarded as well as guarded plots, of equal area, was similar. This agrees with the conclusion of Smith (1938) that, if P guarded plots of a given size excluding guards occupy the same total area as P' unguarded plots of the same size, then the variance within blocks will be equal. Hodnett too arrived at this result.

It is worth mentioning here Smith's (1938) finding that for $b = 0.5$, efficiency is 96% if plots are double or half the optimum size, 80% if plots are quadruple or quarter optimum size, and that the value of b does not greatly effect these estimates in the region of a quarter to four times the optimum size.

Therefore, it appears that unguarded plots of 6.8 to 27.2 basic units are 96% efficient while for those between 3.4 and 6.8 and between 27.2 and 54.4 basic units, the efficiency is 80%. Similarly, guarded plots of size 5.6 to

Table 1

Expressions of wheat yield variation for various plot shapes and sizes

Shape L × W * m × r	No. of basic units	Unguarded Plots			Cf V %	Guarded Plots			Cf V %
		Total No. of plots	Variance of basic unit area	Comparable variance		Total No. of plots	Variance of basic unit area	Comparable variance	
4×1	1	1152	.077	.077	24.7	240	.070	.070	23.9
4×2	2	576	.053	.107	20.6	180	.063	.127	22.6
8×1	2	576	.054	.109	20.8	160	.037	.075	18.8
4×3	3	384	.048	.145	19.5	144	.046	.138	19.6
12×1	3	384	.046	.138	19.1	120	.029	.088	15.5
4×4	4	288	.041	.167	18.2	120	.048	.194	19.9
8×2	4	288	.039	.157	17.6	120	.041	.165	19.2
16×1	4	288	.039	.159	17.8	100	.028	.112	15.1
20×1	5	000	.000	.000	00.0	80	.022	.112	13.9
4×6	6	192	.039	.235	17.6	00	.000	.000	00.0
8×3	6	192	.035	.214	16.8	96	.026	.161	15.7
12×2	6	192	.031	.191	15.9	90	.032	.197	16.2
4×8	8	144	.035	.287	16.8	72	.041	.333	18.5
18×4	8	144	.030	.247	15.6	80	.031	.248	16.8
16×2	8	144	.028	.228	15.0	75	.032	.260	15.9
12×3	9	128	.029	.265	15.2	72	.023	.214	13.9
4×10	10	000	.000	.000	00.0	60	.042	.420	18.4
20×2	10	000	.000	.000	00.0	60	.028	.285	15.4
4×12	12	96	.034	.409	16.4	00	.000	.000	00.0
8×6	12	96	.029	.350	15.2	00	.000	.000	00.0
12×4	12	96	.024	.299	14.0	60	.023	.283	13.7
16×3	12	96	.026	.320	14.5	60	.023	.277	8.9
4×13	13	00	.000	.000	00.0	48	.038	.505	17.9
20×3	15	00	.000	.000	00.0	48	.019	.296	13.0
8×8	16	72	.026	.429	14.5	48	.025	.403	15.1
16×4	16	72	.022	.361	13.3	50	.024	.388	13.9
12×6	18	64	.023	.419	13.5	00	.000	.000	00.0
8×10	20	00	.000	.000	00.0	40	.030	.619	16.8
20×4	20	00	.000	.000	00.0	40	.020	.407	13.1
8×12	24	48	.025	.619	14.3	00	.000	.000	00.0
12×8	24	48	.020	.497	12.7	36	.020	.481	12.7
16×6	24	48	.021	.517	13.0	00	.000	.000	00.0
8×13	26	00	.000	.000	00.0	32	.023	.603	14.7
12×10	30	00	.000	.000	00.0	30	.020	.611	12.8
16×8	32	36	.019	.634	12.5	30	.020	.642	12.7
12×12	36	32	.019	.715	12.5	00	.000	.000	00.0
12×13	39	00	.000	.000	00.0	39	.019	.766	12.7
16×10	40	00	.000	.000	00.0	40	.021	.852	13.0
20×8	40	00	.000	.000	00.0	40	.018	.743	12.5
16×16	48	16	.019	.923	12.3	00	.000	.000	00.0

* L = length in meters.
W = width in ridges.

Table 2

Coefficient of variation per basic unit area for unguarded wheat

Length (1 unit = = 4 m)	Width (1 unit = 1 m)						
	1	2	3	4	6	8	12
1	24.7	20.7	19.6	18.2	17.7	16.9	16.5
2	20.8	17.7	16.8	15.9	15.1	14.6	14.3
3	19.1	15.6	15.3	14.1	13.6	12.8	12.6
4	17.8	15.2	14.6	13.4	13.1	12.6	12.3

Table 3

Coefficient of variation per basic unit area for guarded wheat

Length (1 unit = = 4 m)	Width (1 unit = 1 m)						
	1	2	3	4	8	10	13
1	23.9	22.6	19.7	20.0	18.5	18.5	18.0
2	18.8	19.3	15.7	16.9	15.2	16.9	14.8
3	15.5	16.2	14.0	13.8	12.8	12.8	12.7
4	15.1	16.0	8.9	14.0	12.7	13.0	12.4
5	13.9	15.5	13.0	13.2	12.6	12.7	12.3

22.2 basic units are 96% efficient, while those between 2.8 and 5.6 and between 22.2 and 44.4 basic units are 80% efficient.

Aknowledgement

I am extremely indebted to the staff of the statistics section for technical assistance in the field, collecting and preparing data. My thanks are due to the Director General of the Agricultural Research Corporation for permission to publish this work.

Table 4

Standard error of mean yield of wheat for treatments arranged in randomized blocks (Unguarded plots)

No. of treatments	Size M.×R.	No. of reps	No. of basic units	No. of Plots End to End in Blocks			
				1	2	4	8
4	4×2	144	2	0.026	0.029	0.031	0.000
8	4×2	72	2	0.038	0.041	0.045	0.043
16	4×2	36	2	0.000	0.060	0.063	0.063
4	8×1	144	2	0.030	0.031	0.028	0.000
8	8×1	72	2	0.041	0.045	0.043	0.000
16	8×1	36	2	0.059	0.063	0.064	0.000
4	4×3	96	3	0.043	0.049	0.052	0.000
8	4×3	48	3	0.062	0.071	0.077	0.072
16	4×3	24	3	0.079	0.101	0.111	0.108
4	12×1	96	3	0.051	0.053	0.045	0.000
8	12×1	48	3	0.071	0.770	0.069	0.063
16	12×1	24	3	0.109	0.107	0.103	0.098
4	4×4	72	4	0.058	0.068	0.076	0.000
8	4×4	36	4	0.000	0.098	0.107	0.000
4	8×2	72	4	0.060	0.071	0.064	0.000
8	8×2	36	4	0.090	0.099	0.098	0.000
16	8×2	18	4	0.000	0.148	0.142	0.000
4	16×1	72	4	0.071	0.066	0.000	0.000
8	16×1	36	4	0.097	0.101	0.000	0.000
16	16×1	18	4	0.142	0.141	0.000	0.000
4	4×6	48	6	0.101	0.116	0.135	0.000
8	4×6	24	6	0.161	0.172	0.190	0.183
16	4×6	12	6	0.000	0.222	0.283	0.267
4	8×3	48	6	0.102	0.122	0.108	0.000
8	8×3	24	6	0.148	0.175	0.167	0.000
16	8×3	12	6	0.177	0.251	0.247	0.000
4	12×2	48	6	0.104	0.118	0.102	0.000
8	12×2	24	6	0.156	0.165	0.153	0.133
16	12×2	12	6	0.000	0.246	0.222	0.210
4	4×8	36	8	0.000	0.175	0.197	0.000
8	4×8	18	8	0.000	0.286	0.271	0.000
4	8×4	36	8	0.135	0.165	0.157	0.000
8	8×4	18	8	0.000	0.245	0.230	0.000
16	8×4	9	8	0.000	0.000	0.355	0.000
4	16×2	36	8	0.143	0.145	0.000	0.000
8	16×2	18	8	0.221	0.209	0.000	0.000
4	12×3	32	9	0.186	0.204	0.161	0.000
8	12×3	16	9	0.270	0.293	0.262	0.226
16	12×3	8	9	0.319	0.430	0.393	0.364
4	4×12	24	12	0.176	0.316	0.355	0.000
8	4×12	12	12	0.000	0.392	0.523	0.485
16	4×2	6	12	0.000	0.000	0.700	0.744
4	8×6	24	12	0.061	0.073	0.137	0.000

Table 4 (cont'd)

No. of treatment	Size M.×R.	No. of reps	No. of basic units	No. of Plots End to End in Blocks			
				1	2	4	8
8	8×6	12	12	0.068	0.111	0.202	0.000
16	8×6	6	12	0.000	0.285	0.318	0.000
4	12×4	24	12	0.000	0.136	0.057	0.000
8	12×4	12	12	0.000	0.101	0.085	0.075
16	12×4	6	12	0.000	0.000	0.136	0.116
4	16×3	24	12	0.258	0.245	0.000	0.000
8	16×3	12	12	0.377	0.373	0.000	0.000
16	16×3	6	12	0.430	0.559	0.000	0.000
4	8×8	18	16	0.000	0.452	0.102	0.000
8	8×8	6	16	0.000	0.717	0.000	0.416
16	8×8	3	16	0.000	0.000	0.563	0.704
16	16×4	3	16	0,809	0.000	0.782	0.000
4	12×6	16	18	0.453	0.468	0.373	0.000
8	12×6	8	18	0.494	0.742	0.568	0.494
16	12×6	4	18	0.000	0.913	0.982	0.720
4	8×12	12	24	0.380	0.836	0.731	0.000
8	8×12	6	24	0.000	1.029	0.012	0.000
4	12×8	12	24	0.000	0.715	0.505	0.000
8	12×8	6	24	0.000	0.000	0.895	0.637
4	16×6	12	24	0.639	0.534	0.000	0.000
8	16×6	6	24	0.666	0.939	0.000	0.000
4	16×8	6	32	0.677	0.679	0.000	0.000
8	16×8	3	32	1.386	1.252	0.000	0.000
4	12×12	8	36	0.633	1.304	0.792	0.000
8	12×12	4	36	0.000	1.471	1.586	0.091
16	12×12	2	36	0.000	0.000	1.839	2.231
4	16×12	6	48	0.919	1.591	0.000	0.000

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 417–422 (1991)

WATER USE EFFICIENCY OF PEARL MILLET (*PENNISETUM AMERICANUM* L. LEEKE) GENOTYPES UNDER MID-SEASON MOISTURE STRESS

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(Received: 2 April, 1990; accepted: 16 July, 1990)

Yield and water use efficiency of pearl millet genotypes were significantly affected by genotype, irrigation and their interactions. Irrigation at 25 DAS* and 37 DAS with or without an additional irrigation at 45 DAS showed significant increase in yield and water use efficiency. Yield was positively associated with leaf area, LAI, productive tillers, days to anthesis and days to grain maturation under irrigation conditions. However, both yield and water use efficiency of the genotypes were negatively correlated ($r = -0.99$, $P \leq 0.05$) with the productive tillers. Water use efficiency under irrigation showed positive association with yield, productive tillers and days to anthesis. Improvement of yield and water use efficiency in irrigated pearl millet crop could be due to an increased number of productive tillers maturing synchronously with the main culm due to prolonged vegetative period.

Keywords: grain yield, irrigation, mid-season moisture stress, pearl millet, (*Pennisetum americanum* L. Leeke), water-use efficiency

Introduction

Pearl millet is grown extensively in arid and semiarid tropics as a rainfed crop (Misra 1986, 1987, 1990, Misra and Misra 1990). The severity of environmental inputs limits the yield of the crop (Misra 1987). Higher yields in pearl millet could be obtained by improved breeding and management practices (Ferraris 1973). High yield potentials of crop varieties under adequate management conditions depend upon the full advantage of good moisture conditions (Quizenberry 1982). Short periods of drought, met frequently at critical growth stages of pearl millet, reduce yield considerably (Misra and Misra 1990). Significant gains in grain yield and WUE could be expected in pearl millet (Kanemasu et al. 1984). Pearl millet genotypes can withstand medium to moderate drought conditions during seedling stage (Misra 1990). However, severe growth and yield variations were observed during mid-season moisture stress (Bidinger et al. 1982). Reports on the water use efficiency of pearl millet, which ultimately determines yield of a rainfed crop under variable moisture conditions, are meagre. Further, no report is available on the pattern of water

* Abbreviations: DAS, days after sowing; LAI, leaf area index; WUE, water use efficiency

use efficiency and grain yield under various mid-season moisture stress. In the present study, the grain yield: water use efficiency of high-yielding pearl millet varieties under variable mid-season soil moisture conditions are reported.

Materials and methods

Field experiments with 3 pearl millet (*Pennisetum americanum* L. Leeke) genotypes MBH 1 10, WCC 75 and RCB 2 as main plots and 5 soil moisture (irrigation and rain fed conditions) as sub-plots of 3×4 m^2 with row to row distance of 50 cm and average spacing of plants at 12–15 cm, was conducted at Agricultural Research Station, Durgapura, Jaipur. Each irrigation of 50 mm was provided as shown in Table 2. The climatic data of the experimental site is shown in Table 1. The crop was sown on July 20, 1985. Basal doses of nitrogen as urea 20 kg·ha^{-1} and phosphate as super phosphate 30 kg·ha^{-1} was ploughed under the seeds. Insecticides such as nematocide Thimet 25 kg·ha^{-1} were drilled below the seeds to avoid seedling damage by nematodes during the crop establishment. Weeding was done manually at 20 and 35 days after sowing (DAS). A split application of nitrogen was applied to the crop 10 kg·ha^{-1} after each weeding. Grain yield and water use efficiency were calculated on the basis of individual plants, keeping the plant population at 16 plants m^{-2} in each subplot during the weeding processes. Total water use efficiency was calculated as the cumulative irrigation and rain fall moisture availability to the plots per unit mass of grain yield. Analyses of variance were done for main plot genotypes and subplot irrigation treatments as split plot design with 3 replications in each subplot.

Table 1

Climatic data during crop growth period

Standard Weeks	Rainfall, mm	Relative humidity %		Temperature, °C	
		Minimum	Maximum	Minimum	Maximum
July					
III.	21.04	81	89	22–26	27–34
IV.	30.06	69	84	23–26	30–34
August					
I.	112.03	74	98	23–27	27–33
II.	51.06	88	89	23–26	29–34
III.	51.06	88	99	23–26	31–34
IV.	0.80	60	82	21–26	31–33
September					
I.	0.0	63	79	21–25	30–34
II.	0.0	41	70	21–25	30–36
III.	4.2	48	79	21–25	33–37
IV.	0.2	37	90	21–25	32–37
October					
I.	5.0	37	96	20–24	23–37
II.	10.0	73	96	18–23	23–29
III.	0.0	33	72	14–19	31–33
IV.	0.0	33	47	14–17	31–33

Table 2

Irrigation schedule and respective periods of stress imposed on pearl millet crop. All the genotypes flowered from 41–45 days and matured within 54 to 59 days. The boot leaf emergence was at 25–27 days

Treatments	Irrigation schedule	Stress intervals
I_0	Rainfed (no irrigation)	25 DAS to 45 DAS*
I_1	45 DAS	25 DAS and 37 DAS
I_2	25 DAS and 45 DAS	37 DAS
I_3	25 DAS and 37 DAS	45 DAS
I_4	25 DAS, 37 DAS and 45 DAS	No stress

* DAS: days after sowing.

Results and discussion

Drought stress during the vegetative period is less damaging to pearl millet grain yield than at later growth stages (Misra and Misra 1990). Mid-season moisture stress, between late vegetative or early reproductive to grain filling stage, results in a greater risk than drought injury (Bidinger et al. 1982). The crop growth period during the 1985 monsoon season met with such a stress period (Table 1) leading to a retarded growth and reduction in yield. However, irrigation schedules during boot leaf emergence, flowering and post-flowering stages to overcome these limitations to increase yield, showed that pre-flowering (13) and regular irrigation (14 during the mid-season moisture stress could significantly increase the yield in all the genotypes, varying in their yield potentials (Table 3). The hybrid variety MBH 110 was genotypically superior to the composites WCC 76 and RCB 2 under rainfed and variable moisture conditions. The grain water use efficiency carried similarly with yield (Table 3). The water use efficiency was significantly higher in 13 and 14, compared to rainfed conditions. However, the WUE decreased significantly with stress at 25d through 45d. This might be due to a prolonged period of moisture limitation, severely hampering growth and development of the crop and grain. Sivakumar et al. (1981) showed that regular irrigation at 10d intervals produced the highest pearl millet yield. This treatment is similar to 14.

Dancette (1978) reported that, under marginal water supply conditions, the WUE increased in millets with less water use. However, substantial differences in WUE have been reported in pearl millet in response to irrigation (Ibrahim et al. 1985). The relationship between crop growth, yield and water use efficiency is shown by a regression analysis in Table 4. Under irrigation treatments, the grain yield showed a significant positive correlation with leaf

Table 3

Grain yield and water use efficiency of pearl millet genotypes as affected by irrigation treatments

Irrigation/ Genotypes	Grain yield, mg plant^{-1}			Treatment mean	Water use efficiency mg (mm plant)$^{-1}$			Treatment mean
	MBH 110	WCC 75	RCB 2		MBH 110	WCC 75	RCB 2	
I_0	20.20	12.90	12.0	15.03	112	72	67	83
I_1	19.43	9.23	13.13	13.93	92	44	62	66
I_2	24.77	13.80	17.07	18.55	102	57	70	76
I_3	29.07	18.43	22.27	23.26	120	76	92	96
I_4	32.10	25.53	33.47	30.37	117	93	107	106
Varietal mean	25.12	15.98	15.59	20.23	109	68	80	86
$LSD_{5\%}$								
Irrigation		4.07				12		
Variety		3.57				8		
Interaction		7.07				19		

Table 4

Correlation coefficient (r) values between yield, water use efficiency and plant growth, phenology and yield contributing factors

	Yield g·plant^{-1}	Water use efficiency
Irrigation		
Leaf area, 75d	0.94*	0.85
LAI, 75d	0.91*	0.78
Productive tiller	0.93*	0.97*
Anthesis, days	0.95*	0.92*
Grain maturation, days	0.95*	0.81
Yield, g·plant^{-1}	—	0.92*
Variety		
Productive tiller	−0.99*	−0.99*
Yield, g·plant^{-1}	—	0.99*

* Significant at $P \leq 0.05$ level.

area, LAI at maturity, productive tillers, days to anthesis and grain maturation. However, the varietal yield showed a negative correlation with productive tillers, suggesting that the high tillering habit is not a desirable character under variable moisture conditions for genotypic improvement in pearl millet, although genetically a high tillering potential could favour higher yield under

favourable soil moisture conditions. The water use efficiency had positive correlation with productive tillers, days to anthesis and yield of pearl millet crop under different moisture available conditions. However, the genotypic WUE had negative correlations with productive tillers and a positive correlation with grain yield.

These results suggest that the productive tillers are major determinants of crop yield and WUE in pearl millet. The varietal improvement under a rainfed cropping pattern, however, must depend upon the lower tillering habit which can improve yield and WUE (Egharevba 1977): The tillers contribute less to total yield, but increase the evaporative surface to a large extent. Thus, under variable moisture conditions, they could lead to an adverse relationship with yield, and WUE. To the contrary, increased moisture availability increased the productive potential of the non-synchronous tillers. The developmental plasticity of these tillers contributed to pearl millet yield when irrigated in the pre-through post-anthesis period, avoiding mid-season moisture stress.

Under irrigated conditions, the factor of days to anthesis also plays a significant role in determining yield and WUE. As boot leaf through anthesis stage determines the potential number of Kernels of the mainstem and early tillers (Maiti and Bidinger 1981), adequate moisture availability might lead to a significant increase in the projected productive potentials through efficient water use.

Acknowledgement

The author is thankful to the Director (Res.) of Agricultural Research Station, Rajasthan Agricultural University, Durgapura, Jaipur for providing facilities. The financial assistance of ICAR, New Delhi is gratefully acknowledged.

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 423–433 (1991)

Agricultural education

ROLE OF EDUCATION IN FIELD CROP PRODUCTION

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(Received: 8 June, 1989; accepted: 20 March, 1990)

Several methods have been developed to assess education and demonstrate its importance. We suggest using the number of years spent at school as the measure of education. The extent of education shows the average number of the workers' school years, while the intensity of education indicates the years spent at school per 100 ha. In 1980 the extent of education for those working in co-operative farms was 9.37 years.

There is a significant correlation between education and growing site conditions. Education varies both with regions and production structures. The extent of education is most favourable in the Danube–Tisza Interfluvia, followed by Trans-Tisza and Eastern Trans-Danubia, then by Western Trans-Danubia, but is the least favourable in Northern Hungary.

Although the wheat-, maize- and cattle branches strongly determine the production structure of the agriculture of Hungary, the pea-, sugar-beet- and poultry branches still play an important role from the standpoint of education.

The difference in the intensity of education is much greater (37–350) between the enterprises. It has a decisive role in the per 100 ha fertilizer utilization, value of machines and implements, gross and net production value, and in the volume of earnings and returns.

Keywords: extent of education, intensity of education, growing site conditions, region, production structure, management

Introduction

Out of the qualitative aspects of human resources education, practical experience and various personality features influencing work performance are of decisive importance.

Even Marx pointed out that "the competence of the existing population is the prevailing condition of production, i.e. of economic growth".

The considerable change that has taken place in the professional knowledge of people is today an important element in the utilization of social funds (Bognár 1976).

According to the literary data, studies on the role of education, especially of professional qualification have recently increased in number (Kádár 1980, Pfau 1980, Relin 1980, Isbaner 1980, Sucic 1980, Lehoczky 1980, Bíró 1980, Cselőtei 1980, Dénes 1980).

Nagy (1980) relying on the relevant literary sources uses "professional scoring". In this system the professional scores of a worker range between 0.7 and 2.1. The classification of intellectual workers without professional qualification and of unskilled physical workers is independent of their education.

Besides a system-centered development and enterprisal independence the high professional level of workers and managers is the most important condition for progress (Németi 1986). The qualitative development of the labour force also has an affect on the rapid growth of live labour efficiency (Németi 1986).

According to Van Dijk et al. (1986) the average educational level of population in the USA greatly improved in the fifties and sixties, but this improvement stopped in the seventies. At the time of World War II the American population spent an average of 9 years at school. By the end of the sixties this rose to 12 years, but has not changed since then. In 1980 16% of the population attended high schools or universities for 4 years or more, and 36% graduated from secondary schools, compared to 11% and 31% respectively in 1970.

According to the above data, the average result of verbal/mathematical tests for those born in 1945 was 478/502 points in 1963, while for those born in 1960 424/436 points in 1980. That is, the academic quality of the labour force employed after 1973 was less by 5–6% than previously.

The interaction between technology and labour force is also influenced by the quality of the educational work. The growth rate of productivity gradually decreasing from the end of the sixties in the United States, as well as the low level stabilization of the growth of productivity in the German Federal Republic, France, Japan and Canada, are connected with an undesirable change in the quality of the labour force.

Richonnier (1983) emphasizes the relationship between the new technology and the quality of the labour force. The new technology, while decreasing the quantitative demand, increases the qualitative demand on the labour force.

Hajdú (1987), analysing the interrelations of the technological elements and the spheres and phases of technological planning, established the following:

— out of the factors of technology the technical, chemical and biological factors are inactive;
— change in the agrochemical basis in certain areas is possible almost every year;
— change of technics, cultivars and technology may take place every 4–6–8 years;
— human resources represent the only active factor which mobilizes the elements of technology, ensures the balance, efficient application and improvement of other factors;

— a great contradiction of the human resources is, however, that during the 30–40-year period of active service following the basic education, 3–4 technical changes and 6–8 changes of cultivars may even occur. Thus, without a continuous self-education and systematic extension training it may become a conserving factor.

Therefore, it is not on the size and proportion of the expenses of human resources that their role and importance primarily depend, but rather on how they are able to influence the expenses and efficiency of the technical, biological and chemical factors which fundamentally shape the production cost.

It is, therefore, important to elaborate a method suitable for the objective judgement of the role of these human resources.

Materials and methods

In our investigations we started by postulating a necessary relation between education and the number of years spent at school, considering the quantitative change that assumed a qualitative character.

The number of years spent at school was taken for being equal with the period of education in dayschool. Scores: semi-skilled worker 9, technician 12, college graduate 17, certificated engineer 19.

Categories employed:

— the extent of education is identical with the number of years spent by 1 worker at school,
— extent of higher education: number of years per worker spent in higher educational institutions (university, college),
— intensity of education: number of years spent at school per 100 ha agricultural area,
— intensity of higher education: number of years spent in higher educational institutions by the working staff of 100 ha agricultural area.

The data are grouped by
— growing site conditions (on the basis of gold crown value),
— production regions and
— production structure.

Furthermore, a connection between education and management data was also sought.

In this study, the 1980 balance data of co-operative farms supplied to the Central Statistical Bureau were used.

The computer processing was carried out by Mrs. Szántai in the Computer Centre of the National Planning Board.

Results and discussion

In the co-operative farms the extent of education in 1980 was 9.37 on an average. In some 50% of the 1277 co-operative farms examined this is 9–9.49 (average: 9.26), which is somewhat more favourable compared to the time required to attain a semi-skilled level.

In 18% of the co-operative farms the workers' average educational level is lower than that of the semi-skilled workers. In 31% of them, on the other hand, it approaches that of the skilled workers.

This situation is about twice better compared to the period 30–35 years before, Thus, the co-operative workers have also made a great progress in respect to education.

Table 1 shows the relation between growing site conditions (in terms of average gold crown value) and education. As seen from the table there is a significant relationship between them, except for a single parameter.

Accordingly, unfavourable growing site conditions do not encourage people to attain a higher educational level, nor to acquire professional qualification. Rather, these conditions repel college- and university graduates, and accordingly the ratio of professional workers is essentially lower.

The correlation between education and production region has a similar tendency. The average number of years spent at school by a worker varies from region to region between 8.64 and 9.47. This value is the lowest in Northern Hungary (8.64) followed by Western Transdanubia (9.01) while the data of the other regions (Danube–Tisza Interfluvia, Trans-Tisza, Eastern Trans-Danubia) are close to each other (9.31–9.47) being regarded as the best of all.

This result contradicts the general opinion that Trans-Danubia, and its western part in particular, is the most developed production region of Hungary.

As a result of the educational and economic policy over the past decades, a considerable part of Trans-Tisza and the Danube–Tisza Interfluvia have caught up with the other regions as regards education.

The correlation between education and the level of farm management is indicated in Table 2. The data obviously prove the difference between the semi-skilled and skilled worker level on co-operative farms, in favour of the latter.

In such farms the knowledge required for the reasonable use of increased amounts of fertilizer and for the operation of valuable machines and implements has presumably been acquired by the workers. So in this sense the utilization of materials of industrial origin has raised the level of education usually to such an extent as forced by the objective conditions of farm management. The increase in production value, income volume and profit may also be due to this.

Obviously, workers with a higher level of education do a better quality work both in the organization and implementation of technology and in the organization of production. It is therefore an essential task to shape the personal factors in harmony with the conditions of farm management.

According to the intensity data of education (Table 2) the average number of years spent at school by the working staff of 100 ha ranges from 37 to 475. The difference is more than tenfold. In 46% of the co-operative farms the index for the intensity of education is below 100 (69 on average). In a further 34% this index is between 100 and 150 (average: 125), in hardly

Table 1

Relationship between growing site conditions and education in co-operative farms (1980)

	0–11	11.1–14	14.1–19	19.1–25	25.1–30	30.1–35	above 35	Average	Correlation coefficient *(r)*
	land quality in gold crown								
Number of years spent at school per worker	9.07	9.28	9.36	9.41	9.49	9.51	9.52	9.37	0.92
Number of years spent at school per 100 ha agricultural area	128.74	117.23	140.08	110.92	111.90	134.94	137.83	122.30	0.19
Number of years spent in higher educational institutions per worker	0.42	0.50	0.51	0.54	0.54	0.58	0.52	0.52	0.75
Number of years spent in higher education per 100 ha agricultural area	5.91	6.34	7.57	6.36	6.41	8.22	7.53	6.74	0.65

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Table 2

Relationship between the intensity of education and the cooperative farm management (1980)

	Education indexes of workers per 100 ha								Correlation coefficient (*r*)
	below 37	37–99,9	100–149,9	150–199,9	200–249.9	250–299.9	300–349.9	above 350	
Yield average, t/ha									
Wheat	5.58	4.69	4.85	4.56	5.21	4.61	4.77	4.83	0.28
Sunflower	0.54	1.66	1.71	1.58	1.76	1.19	1.34	1.93	0.46
Maize	2.45	5.46	5.87	5.36	5.23	5.11	5.39	5.09	0.14
Sugar-beet	0.00	36.22	38.60	35.16	39.34	48.65	0.00	33.06	−0.38
Potato	0.00	19.14	18.93	13.96	21.78	18.30	14.89	14.23	−0.49
Pea	0.00	2.51	2.40	2.24	2.52	0.00	0.00	3.20	0.94
per 100 ha									
Worker *n*	4.66	9.01	13.32	19.18	23.60	28.83	38.60	138.05	0.99
Fertilizer consumption, kg	78.06	219.15	237.26	229.93	237.68	249.30	225.23	278.99	0.51
Value of machine implements, Ft	213.49	402.95	505.12	627.55	681.32	764.63	929.80	4109.87	0.99
Gross production value, 1000 Ft	724,38	2324.66	3195.27	4395.94	5554.19	5433.42	8228.26	44015.17	0.99
Net production value, 1000 Ft	145.53	671.21	963.08	1415.37	1848.65	1999.97	3061.56	16022.51	0.99
Field production value, 1000 Ft	371.70	1149.08	1358.64	1425.56	1478.79	1453.41	1584.49	21483.69	0.98
Income volume, 1000 Ft	111.36	557.75	826.84	1180.16	1453.41	1453.36	1515.93	11408.44	0.99
Profit volume, 1000 Ft	2.47	210.36	314.85	438.79	487.51	442.15	1076.95	5167.42	0.99

more than 10% between 151 and 200 (average: 17) and in less than 10% above 200.

No correlation was found between the yield averages of the major crops and the intensity of education. It is only too natural, because compared to the number of workers engaged in crop production the number of those working in other branches is larger by orders of magnitude. It is remarkable that:

— with an increase in the per 100 ha number of workers the intensity of education increases, which suggests that in the Hungarian agriculture of the 1980s the larger per 100 ha working staff means a more versatile production structure and a more intensive production;

— in fertilizer consumption 69 is the average value for the intensity of education, which is a relatively low category. This shows that the higher intensity of education has little effect on a further increase in the volume of fertilizer consumption;

— as opposed to fertilization, the correlation between the value of labour equipment and the intensity of education shows a totally different trend. Here an extremely close correlation ($r = 0.99$) is shown which also means a continuous quantitative change. Qualitative change appears when the intensity of education is below 37, which suggests a low machine- and implement value, or when the education level rises above 300, because in this case the per 100 ha value of machines and implements is more than 4.5-fold of the former;

— the correlation between the other major management parameters examined and the intensity of education shows a trend similar to the former one, but the extent of the difference between farms with an intensity of education below 37 and those where this value is above 300 is more than tenfold for the gross production value, more than twentyfold for the net production value, more than fourfold for the value of crop production, more than twentyfold for the income volume and more than fiftyfold for the volume of profit.

The intensity of education is, besides the intention of the management, primarily an economic necessity, a function of technology, production structure and management strategy, and if it is in accord with the conditions of farming, then it is a positive phenomenon.

If the intensity of education is expressed by the number of years spent in higher educational institutions by the working staff of 100 ha, then this value also shows a positive correlation with the yield averages of the crops examined. In comparison with the former cases this is a new feature (Table 3). How is it possible that on the basis of "the total number of years spent at school" there is no correlation, while on the basis of "the years spent in higher educational institutions" the correlation is positive? Again the explanation is that in an intensive branch of production (such as crop production, compared to the livestock branch) the proportion of "university- and college graduates"

Table 3

Relationship between higher education and co-operative management (1980)

Parameters	Higher education index of workers per 100 ha							Correlation coefficient (r)
	below 2.5	2.51–3.99	4–5.9	6–7.99	8–9.99	10–11.9	above 12	
Yield average, t/ha								
Wheat	4.52	4.58	4.69	4.77	4.98	4.92	5.02	0.84
Sunflower	1.41	1.58	1.66	1.67	1.81	1.93	1.96	0.86
Maize	4.89	5.41	5.56	5.70	5.97	5.65	5.25	0.89
Sugar-beet	35.24	36.68	37.33	36.82	39.27	33.62	39.79	0.49
Potato	12.25	17.62	19.74	18.12	16.48	14.55	17.56	0.35
Pea	1.94	2.31	2.48	2.36	2.62	2.69	2.67	0.72
Per 100 ha								
Workers of higher education, n	8.50	10.00	11.09	13.03	15.43	17.36	59.93	0.95
Fertilizer used, kg	192.56	200.20	231.02	234.40	241.45	257.04	241.45	0.65
Value of machines, implements, 1000 Ft	370.63	368.96	422.01	506.66	568.76	604.09	1845.17	0.95
Gross production value, 1000 Ft	1969.91	2230.10	2692.09	3165.43	3804.60	4314.67	17971.53	0.94
Net production value, 1000 Ft	603.73	630.17	803.65	947.94	1183.11	1335.27	6416.53	0.93
Field production value, 1000 Ft	1035.62	1071.38	1223.89	1315.68	1435.49	1459.75	7969.55	0.91
Income volume, 1000 Ft	521.96	552.73	673.59	801.37	986.60	131.96	4704.71	0.94
Profit volume, 1000 Ft	184.50	198.00	249.53	305.44	361.41	419.71	2058.42	0.93

is a more important factor. Furthermore, the crop production branch of farms usually is managed by workers with higher qualification.

As for the correlation between production structure and education:

— in farms with a poultry branch, regularly higher values were found, unlike those keeping other animal species;

— in the area of crop production, the pea- and sugar-beet branches have an outstanding effect on education, while the same cannot be said of the potato- and sunflower branches. The sugar-beet is well known for its special requirements. The "role" of pea is interesting in this respect. The farms engaged in pea cultivation follow a different production strategy and possess a more foresighted enterprisal management. That is, in these farms the innovative faculty is greater, and so is necessarily the education. In the case of sugar-beet, education is the consequence of the demand raised by this branch, while in the pea branch the higher level of education is a consequence resulting from the occupation.

The extent of education in the agriculture of Hungary today can be concluded from its material and technical level, from the demand raised on agriculture and from the macroenvironment surrounding it. In the technological system based on manual labour and draught power, using primarily materials of agricultural origin, the extent of the workers' education in Hungary showed a value of about 4. In a technological system based on complex mechanization, using masses of industrial origin materials this value is above 11, optimally at least 12 (Hajdú 1987), as supported by the education data and the development process of education in the United States.

In today's Hungarian agriculture the development of technology can be realized by a system that simultaneously ensures

— versatility, i.e. accommodation to changing and differing conditions,
— co-ordination of the technical and agrochemical factors with the environment,
— increased social and operative efficiency of production,
— higher income production and
— sound conditions of market economy.

That is, the scarcity of sources of development, the decreasing or stagnating living standard, and the heavy curtailment of enterprisal incomes must not prevent the development of technology. One of the basic conditions for avoiding difficulties is the renewal of technology. Experiences of the past 20–30 years seem to suggest that can be achieved only with increased investments, higher capacity and more valuable machines and mass application of chemicals. But this is not true. *New ways of the technological development must be found in these days.* The first step should be a *technological development*

linked with the interest relations, and this may be followed by a *market-oriented technological development*. Technical development has a favourable social effect only if it ensures the turning out of products cheaper than before. Finally, a further characteristic of the technical development of our days: *advancing step by step*.

Primarily, not only are new machines wanted, but the existing ones also should be modernized so that with them the same work processes can be carried out with lower energy input and more cheaply. Technologies requiring a smaller quantity and a lower value of agrochemical materials, to ensure the earlier attained production level, should be elaborated and widely introduced.

It is questionable whether the present level of education is sufficient to achieve this goal.

In the future, with personal interest coming into prominence, an enterpreneurial view will be the dominant feature of management. This requires open-minded people, possessing sound basic knowledge, an average European cultural level and familiarity with business relation.

It is a vital social interest to attain a general secondary school level of education.

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 435–450 (1991)

Lectures

IMPACT OF WATER ON SOIL FERTILITY AND ENVIRONMENT

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(Received: 21 September, 1990; accepted: 24 September, 1990)

Introduction

Increasing global demand for and the necessity of the better utilization of commodities have made it imperative to study the impact of major production systems on the environment. Nearing the turn of the millenium we watch with anxiety the development of numerous adverse processes threatening the food and other supplies of future generations. In the issues of agriculture, industry, the communal sphere, as well as of the environment water has become a major concern for the most part of the world. Hydrological conditions, the availability and occurrence of water have distinct consequences for human life particularly in the cases of scarcity or impurity of the water. Water-related constraints limit not only agricultural production but also socio-economic development in many arid countries.

Water conditions and water resources

The sustainability of the environment and society depends to a great extent on the water conditions. The prospects of development also depend on the present and future state and utilization patterns of water resources.

Figure 1 is a schematic representation of the scarcity of water on our globe.

From Figure 1 it becomes clear that only a very little part of the world's water resources is available for agriculture due to the distribution and quality of these resources. This little part, which is fresh water and subsurface water, must be rationally distributed and utilized for the purposes of agriculture, industry, drinking, household, recreation, etc.

Lecture held at "International Conference on Soil Conservation and Environment." Piestany Spa, 29 May–2 June, 1989.

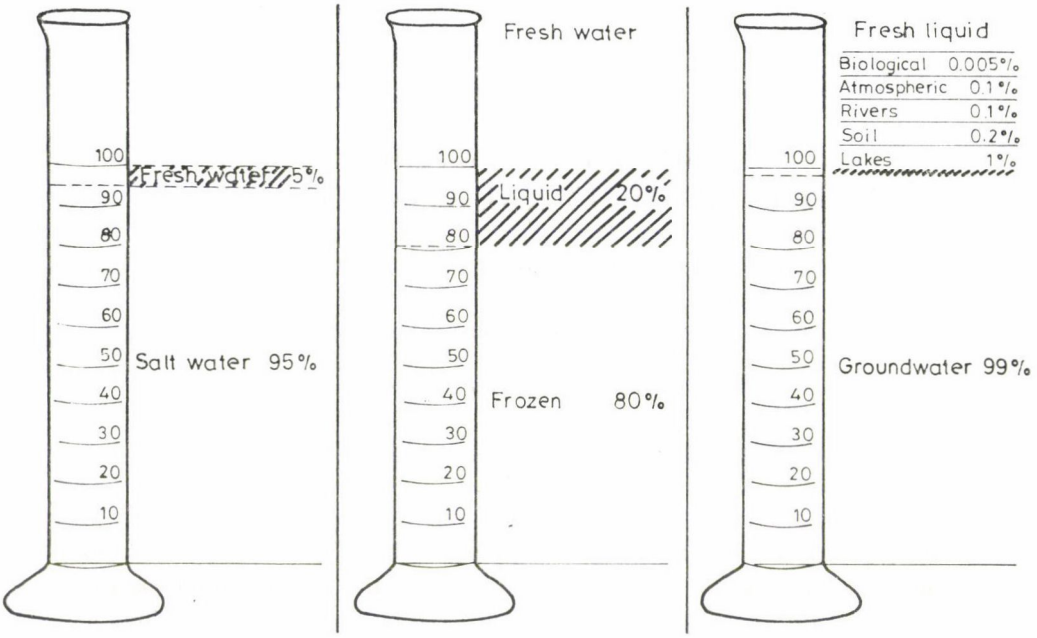

Fig. 1. Approximate global distribution of water

We need a new, holistic approach of scientific and ecological concepts for meeting the demands in respect of the functions, behaviour and future role of water in agriculture with particular regard to the protection and conservation of the environment.

The global water cycle and the natural laws governing this circulation system determine the life on our planet which has the characteristic, quite special in the cosmos, of the presence and availability of water. Based on such considerations we have to agree that water-related problems are not only major environmental problems but also crucial problems of life.

Parallel with the increase of population and the development of civilization water scarcity appeared in many areas of the world, particularly in arid and semi-arid countries, the symbol of which has become the Sahel belt of Africa in the last decades.

When we study the role and importance of water in relation to agricultural production and the environment we have to take into consideration the overall scheme of the water cycle both in nature and production, not forgetting either about the quantities used and polluted in the households. In Figure 2 the action and cycle of water is charted in this perspective after Falkenmark (1988).

Figure 2 clearly shows that with water acting in different branches of agri-, sylvi- and horticulture with remarkable influence on the environment,

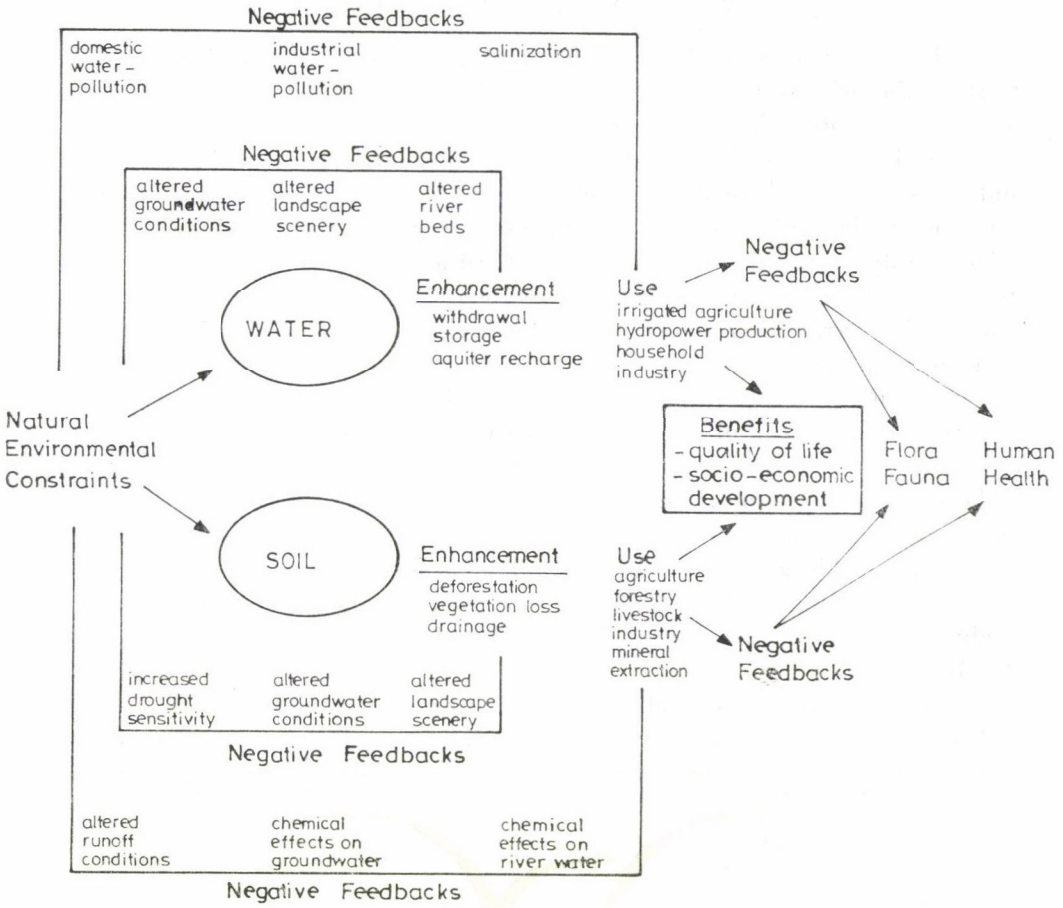

Fig. 2. Linkages between environment, soil, land use and water (after Falkenmark, 1988)

different processes are taking place with effects and feedbacks, including living and non-living systems in close dependence from the pattern of production systems. Different constraints should be taken into consideration and not only cost-benefit analyses but also thorough analyses of the harms and benefits that may come to the environment must be studied before making decisions on higher or lower levels of science and politics on environmental issues.

Figure 2 demonstrates the exceptional complexity of the interaction of water with practically all branches of human activity and all kinds of landscapes and production. Such complexities include processes, as well as politico-social and production conditions. It is obvious that parallel with increasing water utilization the number and nature of negative feedbacks increase and this situation demands very considerate planning, decision-making and production systems developed with circumspection.

Water and sustainable agriculture

In Figure 3, also based on the cited paper of Falkenmark (1988), the partitioning of water on the earth (including atmosphere, lithosphere, pedosphere and hydrosphere) is demonstrated.

As it is impossible to study water use in isolated fields, as in agriculture only, such schemes like Figure 3 must always be taken into account because, particularly in modern society, the fate of water in agriculture is interrelated with the dynamics of water in other natural and/or production systems. The pollution of surface and subsurface waters, and consequently of drinking water with nitrates, or the frequent floods caused by deforestation are good examples of such interrelationships.

When the application of water in agriculture is studied without due regard to the surrounding systems such failure often leads to tragic consequences like the exhaustion of the water resources of adverse changes in the balance of a watershed, the secondary salinization of irrigated areas, man-made deforestation or others.

Sustainable agriculture is a complex question and it is conditioned on a sustainable interaction between water, soil, plants and human activities including all of the ecosystem fed by that cycle (Falkenmark and Lundqvist, 1988).

In the following we shall discuss the role, and impact of water on agriculture with particular regard to irrigation and drainage problems, as well as

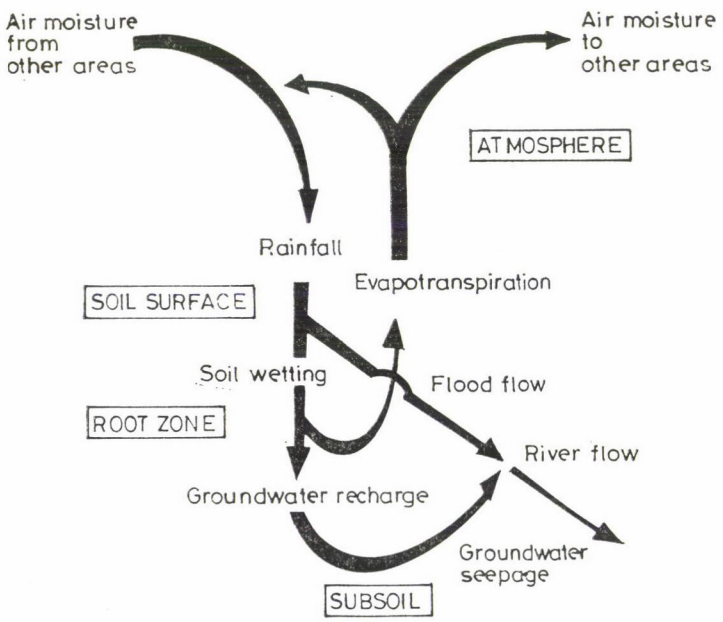

Fig. 3. Water partitioning on earth (after Falkenmark, 1988)

of the changes in soil fertility under the effect, and as a result, of the alteration of water balance in smaller or bigger spatial units.

When speaking of water in respect of agriculture, as well as of sylvi- and horticulture we have practically two aspects in mind.

I. The utilization of water in production under rain-fed conditions

II. Irrigation and drainage.

While in rain-fed conditions the water problem of agriculture is represented mainly by the possibility and regularity of supplying the crops with water from the atmosphere through soils and water resources of subsoil layers in irrigated agriculture, the significance of water is essential and water supplies come mainly from irrigation ditches.

Most of the problems caused by water in agriculture versus environment are related to the second group, namely to irrigated agriculture.

Speaking of water in regard to both non-irrigated and irrigated agricultural production, particularly the first one, related to the problems of drought we have to stress that although drought is partly a meteorological question, nevertheless, must not be neglected or underestimated particularly in a period and area of increasing aridity and desertification. Drought, too, can be induced not only by nature but also by human activities as demonstrated in Figure 4, also based on the achievements of Falkenmark (1988).

Figure 4 clearly shows that aridity may induce desertification resulting sometimes in the collapse of society in cases of overexploiting water resources and the desiccation of land and soil.

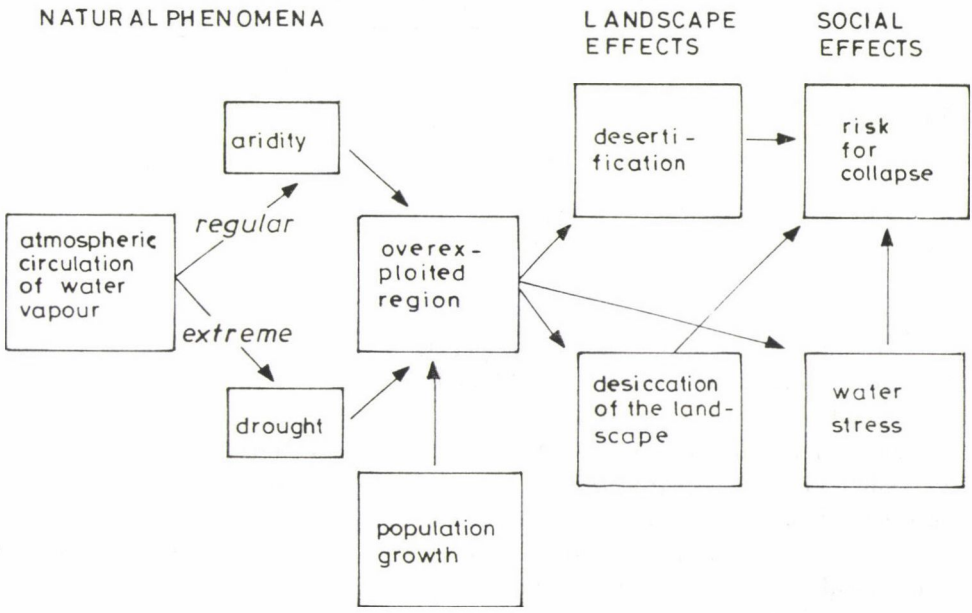

Fig. 4. Various categories of dryness caused by nature and man

Following the considerations described above, we can speak of the past, present and future of irrigation which has been and remains the most important issue in connection with water in agriculture.

A global discrepancy appeared and has become particularly conspicuous in the last decades between the growing demand for water and the increasing scarcity of water resources. This phenomenon has remarkable influence on the future of irrigated agriculture and poses a challenge for science policy to be met before planning further irrigation and drainage systems, as well as during the utilization of the existing ones.

The makers of irrigation plans must reckon with the circumstances demonstrated in Figure 1 and with many other theoretical and practical aspects.

Trends of global extension of irrigation

The expansion of irrigation not only resulted in more areas being irrigated, but also affected the properties of soils, both positively and negatively. Although irrigation dates back to prehistoric times, its rapid development only started about 200 years ago (Table 1).

Table 1

Development of irrigation in the world

Year	Irrigated land (million ha)
1800	8
1900	48
1949	92
1959	149
1988	200

From Table 1 it can clearly be seen that the area of irrigated land grew from 8 million ha in 1800 to 48 million ha in 1900, and more than doubled in the last 50 years. This trend is very remarkable and has resulted in large increases in the production of agricultural products, but has also resulted in a number of technical and environmental problems. Only a few of these will be discussed in this review.

1. At the turn of the century when the irrigated lands of the world did not exceed 50 million hectares, they were mainly distributed among a few dry countries, as shown in Table 2.

At the beginning of the century the countries listed in Table 2 accounted for 2/3 of all the irrigated soils of the world. At that time irrigation was only practiced in dry regions. However, in recent times, irrigation has been extended into many semihumid regions, as well as arid and semiarid regions. The problems of salt accumulation and secondary salinization are different among different climatic regions with respect to the chemistry of salt accumulation.

Table 2

Irrigated soils in some dry countries in 1900

Country	Irrigated territory (million ha)
Indian subcontinent	15.49
Russia	3.80
United States	3.01
Japan	2.72
Egypt	2.00
Italy	1.30
Spain	1.00
Chile	0.30
	Total: 30.13

2. In many countries irrigation has been introduced as a new practice within the last few decades. Experiences of countries having long-term irrigation practices were not always known and applied to combat against secondary salinization.

3. The extension of irrigation affected not only the land actually irrigated, but also the neighbouring non-irrigated territories. As long as irrigation was concentrated in small areas, evidently its environmental effect was much less than that of big irrigation systems that affected large surrounding areas.

4. The effect of irrigation on the biosphere (besides the irrigated crop), increased parallel with the sharp development of this method including adverse effects, such as

— salinization and contamination of drinking water
— water-logged and saline areas as a breeding ground for parasites and diseases
— toxic effects on soil microorganisms, etc.

The listed phenomena, and others, constitute in many places a barrier not only for further development of agriculture and human civilization but also keeping the present level of production.

Effects of increase of irrigation on salt accumulation in soils and waters

The increase of irrigated territories in different parts of the world and in different countries and regions has been remarkable. In the USA, for example, the area under irrigation doubled between 1949 and 1973 to 21 million hectares. Another example: in the USSR every year 1 million ha of new irrigated land are brought under cultivation. In Kenya the area of irrigated land doubled between 1959 and 1969, and further development is envisaged. In Hungary irrigated areas have shown a more than 10-fold increase since the Second World War ended in 1945.

In many countries where irrigation was introduced mainly under non-arid conditions in densely populated areas, its side effects were different from those appearing in most of the arid countries. For example, in dry countries the area surrounding massive irrigated lands is a vast desert which makes possible to tolerate the adverse consequences of irrigation such as secondary salinization of adjoining areas (Jegorov 1969, Kozlovsky and Kornblum 1963). In countries where the utilization of land is over 70%, like in Hungary, the above-mentioned and similar side-effects would be catastrophic.

In some countries, like Egypt, nearly 100% of the agricultural land is irrigated (Elgabaly 1962). The corresponding figures are: 70% in the Malgache Republic, 26% in Thailand and 50% in Pakistan (Nazir Ahmad 1965). Similar ratios exist in many arid and semiarid countries. In less arid or semihumid countries the irrigated land often accounts for only a few percent but this percentage is sharply increasing even in those countries. For example, in 1980, nearly 13% of the total agricultural land was irrigated in France, more than 10% in Spain, and nearly 15% in Greece (Servant 1969).

In spite of the availability of many sources of information, accurate data concerning the lands of the world where irrigation has recently been introduced are very scarce. Widely different accounts and estimates can be found in various papers and records. Estimates of total irrigated lands in the world range from 150 to 250 million hectares. The explanation for such diversity of information is probably the fact that it is one thing to register the existing irrigation systems in the world and it is another to keep record of the ones which are in permanent operation. This is the reason, in all probability, why the data of FAO (Food and Agricultural Organization of the United Nations) and ICID (International Council for Irrigation and Drainage) are always different regarding the area of irrigated land.

It is evident that the neglected or abandoned irrigation systems are rather frequent and account for a very high percentage of all existing systems. According to the estimates of FAO and UNESCO (United Nations Educational, Scientific and Cultural Organization), as much as half of all the exist-

ing irrigation systems of the world are more or less under the influence of secondary salinization, alkalization and water-logging. This phenomenon is very common not only in old irrigation systems but also in areas where irrigation has only recently been introduced.

According to the estimates of all of the above-mentioned agencies, 10 million hectares of irrigated land are abandoned yearly as a consequence of the adverse effects of irrigation, mainly secondary salinization and alkalization.

The mentioned losses and damages are not evenly distributed among the irrigating countries. In some of them the damage is low, but in others it can be as high as constituting a major problem in the agriculture or even in the national economy of the country. Unfortunately, the world is rich in such sad examples. In Pakistan Nazir Ahmad (1965) carried out statistical analyses in respect of secondary salinized land. According to his data, out of 13.8 million hectares of total irrigated territory, salinized areas accounted for 2.1 million hectares after a few years of irrigation. He indicated among the causes of secondary salinization in Pakistan the joint effect of irrigation and ground water. It is known from FAO reports (1976) and the papers of Kovda (1980) that more than 50% of irrigated soils in Iraq and Iran is affected by secondary salinization.

According to FAO estimates (FAO 1971, 1972) for Iraq, 50% of the river plain is affected by salinity. The same authors estimate the extension of salinity and alkalinity in Iran to be more than 15 percent of total land surface, exceeding 25 million hectares. As a result of irrigation, the salinity problems are increasing year by year.

In Turkey more than 25% of alluvial soils are already affected by salinity and practically all of them may become saline due to the envisaged increase of irrigation (FAO 1971).

FAO reports (1971) on salinity in Syria in the Euphrates-Valley and estimates the adverse effects as follows:

(a) For more than 20,000 ha salinity developed to a level where these soils had to be taken out of cultivation, and the loss is estimated at a total of 30,000 tons of cotton *(Gossypium hirsutum)* per year.
(b) For about 30,000 ha the yield decreased by 50%, and the total loss is estimated at 20,000 tons of cotton per year.
(c) For about 60,000 ha the yield decreased by 20%, and the total loss is estimated at about 18,000 tons of cotton per year.

The Near and Middle East region depends largely on irrigated agriculture. Although only 36 percent of the arable land of the regions is irrigated, this area produces 70 percent of value of all crops. It is envisaged that irrigation by the next decade will increase to 51 percent of the arable area. However, the problem of salinity is also expected to increase on these soils because they have a high potential for becoming saline (FAO 1971).

At present no continent is free from very serious occurrences of salinization and alkalization. According to Zavaleta (1965), practically all irrigated alluvial soils in Peru show the features of salinity and alkalinity. In Argentina 50% of the 40,000 ha of land irrigated in the 19th century are now salinized (Kovda 1980). In Australia secondary salinization takes place in the valley of the River Murray and Northern Victoria where some 80,000 ha have been affected (Loveday 1985, Jenkin 1981, Northcote-Skene 1972). The same phenomena can be observed in Alberta, Canada (Clayton et al. 1977). Similar processes have been recorded in the northern states of the USA where irrigation was introduced much later than in the dry west. It has to be noted that the last four examples and many other irrigated regions are far from being arid and the majority of salts accumulating are associated with the sodium salts capable of alkaline hydrolysis and not with the neutral sodium salts we are familiar with in desert and semi-desert areas (Grin 1962, Malcolm 1982, Munteanu-Ionescu 1964, McIntire et al. 1982).

A great number of sources are available referring to the different problems of secondary salinization and alkalization and describing their adverse effects in many countries. Based on the literature, the following countries can be mentioned where the salinization and/or alkalization of irrigated soils either represented a serious problem in the past or poses such problems at present. In Europe: Austria (Husz 1965), Bulgaria, Czechoslovakia, Cyprus, France, Greece, Hungary, Italy, Portugal, Rumania (Obrejanu 1964, Sandu 1966), Spain, the USSR (Pekatoros 1962, Novikova 1967) and Yugoslavia (Zivkovic 1965, Miljkovic–Plamenac 1971, Szabolcs 1979), in North America: Canada and the USA (Richards 1954), in Mexico and Central America: Cuba and Mexico, in South America: Argentina, Brazil, Chile, Columbia, Peru and Venezuela, in Africa: Algeria, Angola, Chad, the Cameroons, Egypt, Ethiopia, Ghana, Kenya, Libya, Morocco, Niger, Nigeria, Somalia, South West Africa, Sudan, Tanzania, Tunisia, Zambia and Zimbabwe, in Near and Middle East and South Asia: Afghanistan, Burma, India, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Pakistan, Saudi Arabia, Syria, the Trucial States, Turkey and Yemen, in North and Central Asia: China, Mongolia and the USSR, in South East Asia: Indonesia, Malaysia, Thailand and Vietnam, and in Australasia: Australia (Peck et al. 1983, Pels and Stannard 1977). According to the knowledge of and the data available for the author, in these countries secondary salinization not only occurs, but represents practical problems. The absence from the list of several countries where the phenomenon may also occur is due to missing records or the lack or information of the author of this paper (Szabolcs 1979).

Nearly 1/10th of the continents of our globe is touched with soil salinity and/or alkalinity. A certain part of this territory is under irrigation but the greater part of irrigated areas consists of soils which are non-saline and non-alkali at least for the moment. Part of such land can be potentially saline or potentially alkaline. Potential salinity or alkalinity means that a soil which is neither saline or alkaline at the moment can be turned into either by applying improper methods of irrigation. Most of the present secondary salt affected soils have gone through this process (Szabolcs 1979).

While we have reliable records on the extension of salt affected soils on our globe, unfortunately we are still lacking proper data on the world extension of secondary salinized or alkalized soils, we have to make our own estimates.

Neither do we have proper data and records on the extension of potentially salt affected soils. It is necessary first of all to clarify this term. As mentioned above, potential salt affected soils are those, which are non-saline or alkaline on the top layers at the moment, but may be salinized due to irrigation. Evidently, such a definition is relative because any soil can be salinized, e.g., even irrigated with saline water or in lack of any drainage (Talsma 1963).

The ground-water plays a significant role in salinity due to seepage from channels during the irrigation. From unlined canals large amounts of irrigation and drainage water seeps into ground-water lifting the water table near to the surface of irrigated and adjoining soils. There are situations where this contribution to ground-waters may be as large as and sometimes even more than the quantity actually utilized by irrigated crops.

This new accretion to ground-waters may be withdrawn from the subsoil, wholly or in part, by wells or tube-wells. Under good subsoil drainage conditions, it may seep into rivers. Under other conditions, it may raise the subsoil water level or fill pockets or form perched water tables and cause local waterlogging necessitating drainage for restoring healthy conditions in the soil.

Consequently, irrigation also changes the salt content and salt distribution in the soil profile (Szabolcs 1979, Szabolcs and Darab 1968). That is why the definition of secondary salinization and alkalization should always be examined against the background of the methods or irrigation, soil and water properties, farming pattern, natural of artificial drainage, etc. This is the reason why the diagnosis of secondary salt affected soils based on a simple soil survey is always difficult and often omitted even during the planning stage of irrigation. This omission has caused a lot of unexpected harm in the first years or later periods of exploration of irrigation systems in many countries of the world. Evidently, under different climatic conditions secondary salinization has different interpretations. Closely related to the biogeoche-

mical processes of salt accumulation, evidently the hazard of secondary salinization is greater in desert areas than in humid regions where natural leaching processes remove soluble salts.

Survey and estimation of possible potential salt affected soils were carried out in some European countries (Szabolcs 1974). The figures are shown in Table 3.

Table 3

Existing and potential salt affected soils in some European countries

Country	Salt affected soils (ha)	
	Existing	Potential
Austria	500	500
Czechoslovakia	26,000	80,000
Hungary	740,000	885,000
Italy	500	400,000
USSR	28,000,000	18,000,000

The data of Table 3 clearly demonstrate that even in those European countries where the hazard of salt accumulation is far less than that for some countries discussed earlier, the area of potential salt affected soils is similar or greater than the area of recent salinization. Evidently, under arid conditions, this ratio will be even higher.

In many arid and semiarid areas practically all soils or at least a high percentage, can be termed as potentially saline. As a consequence of the above described regularities, the determination, grouping, characterization and mapping of secondary salt affected soils must be performed in the context of the local environmental and economic conditions (Ianovici and Florea 1964).

Man-made salinization is a way to the destruction of the global biospheric mechanism with an influence not only directly on the soil but also indirectly, on several processes from photosynthesis to the cycling of bioelements (C, O, N), etc. Such influences must also be taken into account with respect to the soil organic matter, energy resources, and soil bioprocesses, etc. The negative consequences of soil salinization are not only social and economic; they are globally destructive for the biosphere of our planet (Kovda 1980).

Combat against secondary salinization and alkalization

The extension of irrigation remains a major prospect for increasing yields and for nourishing the world's population. If not only the explotation of irrigation systems is done carefully, but also during the planning and construc-

tion phases of irrigation systems the necessary preliminary surveys and precautions are carried out and taken into consideration, respectively; then the production of food and raw materials can be multiplied in the future on a world-wide basis.

Many prognoses are available with respect to the development of irrigation for the turn of the millenium and for the 21st century. Part of them are local or country reports, but some of them are on a global scale, like the well-known Report for the President, ICID prognoses, etc. (Aleksevsky 1974). It is evident that in different sources different figures can be found, but on the average about 400 million ha of irrigated land are predicted for the first part of the 21st century. Unfortunately, no reliable predictions are available on the hazard of the development of secondary salinization resulting from such a sharp increase of the territory of irrigated land in the near future. Based on experiences, we have to agree that in general the increase of the hazard of secondary salinization and alkalization is not in linear proportion with the increase of the acreage of irrigated land. The correlation is closer to logarithmic. We are still lacking the exact analysis of the rate of the possible hazard of recent and predicted extension of irrigation in different countries (Szabolcs and Darab 1982).

Evidently, it must be very diverse in the different areas, regions, and districts, but if secondary salinization increases in space and extent as a world-wide process, it is more than probable that its global importance will sharply increase in the future.

It is relevant to develop, whenever possible, and to adopt different studies and their results in the local, national and even international planning of new irrigation systems as soon as possible. It is also necessary to extend such studies to the predictable joint effect of existing and future irrigation systems on the environment.

In case of future development of irrigation not only the pedological but also the general environmental influence of irrigation will assume new dimensions. For instance, the concentration of CO_2 in the atmosphere, and its rapid change affected by human activities, is a widely discussed problem of our days. A great number of books, reports, and prognoses are available on this subject; some of them threatening with consequences which would be tragic for mankind. If the territory of irrigated land doubles or trebles, the irrigated plants will be able to produce higher amounts of biomass and harvest, through photosynthesis and to consume as much as 30–40 billion tons of CO_2 annually, instead of the recent 15–20 billion tons (Kovda 1980).

The technical literature presents many examples that clearly show that the successes of irrigation are also interrelated with numerous vital problems. As it is clear from the mentioned example, the aim of irrigation development is to improve the food situation, as well as the environment. It is also evident

that the hazard of secondary salinization and alkalization will be one of the major obstacles in the way of this development if we do not intensify the study of this risk and apply the methods for its prediction and prevention.

Preliminary survey and control of irrigated soils

In Table 4 a scheme of methods, recommended for the control of salinity and alkalinity in irrigated areas is given.

This table shows that the prediction of secondary salinization and alkalinization of the soils to be irrigated should be based on a preliminary survey of the landscape and soils before the construction of the irrigation system. In this way, it is possible to take the necessary steps for the prevention of adverse processes.

During irrigation, a well-organized monitoring of the soil and water properties is to be conducted in order to record changes, if any, and to underlie taking precautions, if necessary. Monitoring methods as well as the timing and location of sampling depend upon local conditions.

In the course of making the survey and monitoring, it is necessary to develop a reliable method for the prediction of salinization and alkalization.

Table 4

Scheme of methods recommended for the control of salinity and alkalinity in irrigated areas

(A) Before construction of irrigation system		*Preliminary survey*
	Landscape	*Planned irrigation*
	climate	available irrigation water quality and quantity
	hydrology	ground-water depth and quality
	hydrogeology	technology of irrigation
	geomorphology	cropping pattern tolerance
(B) During irrigation		*Monitoring*
	salinity and alkalinity of soil and ground-water table	
	chemical composition of ground-water	
	chemical composition of irrigation water filtration	
	physical soil properties	
	toxic elements, if any, in soil and water	

The mapping of the results of preliminary and subsequent surveys constitutes not only a good display of soil and environmental conditions of the irrigated areas, or areas to be irrigated, but also guidelines for proper

irrigation and land protection. Such systems elaborated by various authors, for different places and conditions are also available in technical literature (Szabolcs et al. 1969/a, b).

The monitoring system of irrigated areas must be elaborated and/or adapted and also closely related, to local circumstances. Soils, irrigation water, and ground-water must be studied regularly and whenever discrepancies occur with the predicted salt regime, the necessary measures should be taken either by diminishing the acreage or the intensity of irrigation or by improving the drainage of the land.

We must rationally utilize, and at the same time, save our water resources because it is not only agriculture but also the fate of forthcoming generations and that of the whole biosphere which depends on the proper conservation and future availability of the world's water resources.

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Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 451–452 (1991)

Obituary

OBITUARY OF JÁNOS DOHY

The older agriculturists, and especially the phytopathologists were shaken by the news that Dr. JÁNOS DOHY professor, the outstanding expert, excellent pedagogue, a highly respected man who led an exemplary life died on 6th March of this year. With his departure, a valuable life starting in Kolozsvár on 19th October, 1905 ended. From his 85 years, almost 65 were equally rich in success and trials. He accepted the successes with modesty and the trials with wise resignation. Dohy came from a teacher's family; his father retired as a department leader at the Pallag Agricultural Academy. After his elementary and secondary school studies, completed in Kolozsvár and Debrecen, he acquired a diploma with distinction in 1926 at the Debrecen Agricultural Academy. Three years later he was already a professor's assistant working at the Botanical Department of the Magyaróvár Agricultural Academy. He took his doctor's degree in 1930 at the Kossuth Lajos University, Debrecen. In 1938 he was appointed to an assistant academic professorship, then in 1940–1944 became department leader professor in the Agricultural Col-

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lege, Kolozsvár. After the war, until 1949 he was head of the Phytopathological Department in the Debrecen Section of the Agricultural University. With the reorganization of the agricultural higher education he was transferred to the Plant Breeding Station at Kisvárda, which he accepted with resignation. In 1954 the Agricultural Academy at Mosonmagyaróvár was reopened, and Professor Dohy, besides heading the Departments of Botany and Zoology, also attended to the sub-rector's duties. After 1957 he was convicted on the basis of the historical judgements of those days and was incarcerated for nearly 5 years. Even in 1962 he was only given a humble post through the good offices of the managers of the day at the Lábodi State Farm. From there he was taken over by the Keszthely College of Agricultural Sciences and was employed at the potato breeding plant of Rinyatamás for 3 years; in 1969 he retired from his Keszthely working place as a scientific administrator. Professor Dohy accepted this series of tribulations with patience, during which he received great support from his loving wife and children.

In appreciating Professor Dohy's scientific activity others are competent; this year's Széchenyi Prize duly suggests that he did valuable, successful work. Alas, only his son (a professor himself at the Gödöllő University of Agricultural Sciences) could accept the Prize on his father's behalf, because by then János Dohy was no longer among us.

I first met Professor Dohy in 1942 when I became a student at the Argicultural College, Kolozsvár. We listened to his lectures with reverence; they were clear, logical and supported by highly expressive drawings. As a teacher he was strict and methodical, but understanding, always pleased with the student's knowledge and success. During my career I knew Professor Dohy as an excellent superior too, from whom I learned at least as much humanity as profession. He had extraordinary sense to give ideas; if I was uncertain in some question he could suggest 3–4 variations, and the next day was glad if I chose well; that is how he trained me to think. His life, his bearing, his activity shaped me to a considerable extent, he was and has remained one of my ideals. Since he taught over many years, his life and bearing became a model for many generations. We remember him with great respect, for those whom he taught his way of living and working will always be an example.

I. VINCZEFFY

Acta Agronomica Hungarica, Vol. 40 (3–4), pp. 453–455 (1991)

Book reviews

Statistical methods for genetic improvement of livestock. Edited by D. GIANOLA and K. HAMMOND. Advanced series in agricultural sciences 18, Springer-Verlag, 1990

With this book the reader acquires the 18th volume of the "Advanced series in agricultural sciences" published on 534 pages by the SPRINGER CONCERN under the editorship of the excellent American and Australian scientists. The bulk of the volume consists of the lectures delivered at the international symposium held in Armidale, Australia 16–20 February 1987. The twenty-two discussion-leading co-editors are the best representatives of the profession from all parts of the world. Seven similarly excellent experts were invited to write summaries of the subjects of the discussion and content of the book.

Part I. deals with general statistical questions; first of all a historical review is given of the statistical methods of livestock breeding. Further major subjects are: methodology and box-cow theory; models on the distinction between the alternative ways of inheritance; evaluation of inbred lines, their F_1 generations and back-crosses; polygenic inheritance, regressive models, genetic hypotheses.

Part II. discusses animal experiments and breeding programme planning. Major subjects are: regression and genetic correlation between parents and progenies; planning of single- and multigeneration selection experiments; estimation of the efficiency of genetic variance and selection; optimum plans concerning the methods of breeding value estimation.

Part III. has the heading "estimation of genetic parameters". Mathematical–statistical questions of determining the genetic variance on the basis of the theory of probability; ANOVA variance analysis and ways of its expression; methods of probability calculation; REML-system estimation method; NEWTON–RAPHSON algorhythm; logarythmic calculus of probability; linearization; methods of evaluation by points; EM-algorhythm and method of successive approach; confidence intervals and hypothesis tests; examples of the application of these methods — are the major subjects of this chapter.

Part IV. deals with the forecast and estimation of genetic qualities. Mixed linear models are described. Further subjects: the question of known and unknown variance factors, prognostication of future records; description and evaluation of the BLUP and BEYOND method.

Part V. acquaints the reader with methods of estimation and prognostication through linear and non-linear models. Major subjects: role and use of standardized linear models in livestock breeding; estimation of heritability by analysis of regression between parents and progenies; gene frequency estimation; analysis of linear and non-linear growth models by random parameters; construction of non-linear growth curves; evaluation of survival, resistance and other properties and observations in livestock breeding; expression of survival and resistance, determination of

their extent; parametric and semiparametric models.

Part VI. deals with some questions of selection. BLUE- and BLUP method used in selection work; right choice of female animals (cows); equations to balance genetic and ecological trends; assessment of the genetic value of father and progeny in evaluating the father; problems of grouping and of certain special treatments in judging the breeding value of males; problems of the matrix of relation used in livestock breeding; additive genetic variance; examples of using the method of NRM (non-random-mating); evaluation of progenies of unknown origin in the case of using NRM.

Part VII. deals with the determination of highly effective genes. Subjects of special interest: question of the number of genes; segregation in crossing and backcrossing; segregation analysis; repeated back-cross and selection; use of markers; physiological markers; population analysis; methods suitable to seek out major genes; reproductive technology, additive gene effect; embryo transfer, embryotomy, embryo- and spermium chimaeras, polyploids, gene transfer; evaluation of the effect of non-additive genes, cytoplasmic inheritance, dominance effect, preference treatments.

I. HEROLD

Plant Pathogenic Bacteria Part "A" and "B" (Proceedings of the 7th International Conference on Plant Pathogenic Bacteria, Budapest, Hungary, June 11–16, 1989.

Among plant pathogens hypomycetes and viruses that cause the greatest losses of yield are followed in order by bacteria. Although this order truly reflects the present volume and economic importance of the damages, sometimes and in some places pathogenic bacteria cause very serious losses (e.g. in Eastern Hungary the appearance of Streptomyces scabies in potato resulted in a 30–40% yield loss at the end of the 1980s.

Therefore an international assessment and evaluation of experiment results concerning plant pathogenic bacteria, as well as the subsequent setting of further tasks at least every third year, are not only reasonable but also indispensable and extremely useful.

The 7th International Conference on Plant Pathogenic Bacteria was held on 11 June 1989 in Budapest, Hungary, organized by the International Society of Plant Pathology–Bacteria Section and the Hungarian Association of Agricultural Sciences, under the auspices of the Hungarian Academy of Sciences, the Hungarian Ministry of Agriculture, the Hungarian Society of Microbiology, the Hokko Chemical Industry Co., Ltd., Japan and the International Society of Molecular Biology.

From the material of the Conference 171 papers (including the material of the four plenary lectures), the production of 483 authors, have been published by the Hungarian Academy of Sciences in the Proceedings, under theeditorship of Z. Klement.

The opening lectures: New trends in phytobacteriology (Kelman, K.), Recent trends in biochemical and physiological aspects of bacterial plant pathology (Goodman, R.N.), Strategies for restraining bacterial plant disease (Rudolph, K.), Use of genetic and molecular approaches in elucidating bacterial plant pathogeneticity determinants (Boucher, C. and Arlat, M.) show — among others — what a great increase in the number of lectures (publications) on phytobacteriology took place from 1966 to 1988, e.g. in the scientific sessions of the American Phytopathological Society.

The lectures also indicate how many experts in biochemistry, molecular biology and genetics have been drawn to phytobacteriological research work by the biotechnology.

The new techniques (genetics, computer technics, etc.) have greatly increased the capacity and success of research. A comprehensive view is given of the causal relation between chemotaxis and pathogeny, of the achievements of studies on toxins, bactericides, antibiotics, biological control, resistance breeding, and on the molecular bases of pathogenic specificity.

The papers may be limited in length, but up-to-date relevant references cited and listed

in them — more than 1500 passages — provide further information to those interested in these various subjects.

The subjects of the lectures generally represent a valuable contribution to the present knowledge of phytobacteriology, and not only enrich its theoretical results but also promote their application in practice.

New directions are set and practical methods marked out for molecular biology (molecular genetics) and biotechnology, and those working in these fields or engaged in bacteriology-phytobacteriology register the cooperation as an everyday task.

Besides phytopathology (phytobacteriology) general and applied microbiology (bacteriology) have also been made richer with many new data by the conference and by the publication of its material.

In accordance with the structure of the Conference — with the answers given to the questions circulated through the Conference taken into consideration — in the two volumes of the Proceedings (Part A and B) the material of the lectures delivered are grouped in 9 sections plus a mixed part, running to a total of 1061 pages with 189 tables and 232 figures. In the sections following the lecture held in the plenary session, 26 papers represent the host-parasite relationship, 14 the control and biocontrol, 12 the epidemiology, 20 the genetics and molecular biology, 36 the identification, 25 the erwinia, 7 the agrobacteria, 14 the serology and 15 papers represent lectures on various subjects.

The book can be of good use for phytobacteriologists, bacteriologists, microbiologists, and for those working in the field of phytopathology and phytopathological physiology. Valuable data can be found in it by those engaged in molacular genetics (microbial genetics) or biotechnology (microbial and plant biotechnology), too.

This book is important as to research workers and teachers, and also to university students dealing with special subjects, it can equally be recommended for libraries of universities and research institutes.

M. KECSKÉS

REVIEWERS OF MANUSCRIPTS, VOLUME 40, 1991

Every scientific contribution in Acta Agronomica Hungarica is reviewed by two scientifically qualified persons. The Editorial Board is pleased to publish the following list of reviewers for the manuscripts of the 1988 issues, who by their unselfish contribution have significantly contributed to ensure the scientific standards of Acta Agronomica Hungarica.

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PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat, Budapest

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GENERAL INSTRUCTION

Two copies of the manuscript and two sets of the figures should be submitted to:

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Manuscripts in English or in Hungarian including Abstract, References, Tables and Legends should be typed double-spaced (25 lines, 50 characters per line including spaces) and supplied with authors' names, page number. Tables should be on separate, numbered pages after the References. Legends for figures, on a separate page, should follow the tables. Standard articles should not exceed seven pages.

FORMAT

Title. The title should reflect the most important aspects of the article, in a preferably concise form of not more than 100 characters and spaces.

By-line. The authors' names should be followed by affiliations and addresses. (No inclusion of scientific titles is necessary.)

Abstracts are required for all the manuscripts. They should be typed in one paragraph and limited to max. 200 words. Below the abstracts, an alphabetical list of keywords should be given.

Text. Major sections after the introductory statements are: *Material and methods*, *Results*, *Discussion*, *References.* Subheadings may be used, though the unnecessary fragmentation of the text should be omitted.

Style. After acceptance for publication, manuscripts are reviewed for style, grammar and clarity of presentation.

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Authors can facilitate editing work by indicating in pencil, the precise meaning of certain symbols (e.g.: distinguish O from zero, the number 1 from the letter "l", the multiplication × from letter X).

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Zinovev, L. S., Matalova, T. S. (1976): Protaviteli, bezopasnie dlya klubenykovykh bakterii. Zashchita Rastenii, **5**, 29–31.

Mather, K. and Jinks, J. L. (1971): Biometrical genetics. Chapman and Hall Ltd., London, U. K.

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